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(51) International Patent Classification 5 :		(11) International Publication Number:	WO 92/03568
C12P 21/06, C07H 15/12 A61K 31/70	A1	(43) International Publication Date:	5 March 1992 (05.03.92)

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(54) Title: SUGAR MODIFIED OLIGONUCLEOTIDES THAT DETECT AND MODULATE GENE EXPRESSION

(57) Abstract

Compositions and methods are provided for the treatment and diagnosis of diseases amenable to modulation of the production of selected proteins. In accordance with preferred embodiments, oligonucleotides and oligonucleotide analogs are provided which are specifically hybridizable with a selected sequence of RNA or DNA wherein at least one of the 2'-deoxyfuranosyl moieties of the nucleoside unit is modified. Treatment of HIV, herpes virus, papillomavirus and other infections is provided.

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SUGAR MODIFIED OLIGONUCLEOTIDES
THAT DETECT AND MODULATE GENE EXPRESSION

FIELD OF THE INVENTION

This invention relates to the design, synthesis
5   and application of nuclease resistant oligonucleotides
which are useful for antisense oligonucleotide
therapeutics, diagnostics, and research reagents. Sugar
modified oligonucleotide which are resistant to nuclease
degradation and are capable of modulating the activity of
10 DNA and RNA are provided. Methods for modulating the
production of proteins utilizing the modified
oligonucleotide of the invention are also provided.

BACKGROUND OF THE INVENTION

It is well known that most of the bodily states
15 in mammals including infectious disease states, are
affected by proteins. Such proteins, either acting
directly or through their enzymatic functions, contribute
in major proportion to many diseases in animals and man.

Classical therapeutics has generally focused upon
20 interactions with such proteins in efforts to moderate
their disease causing or disease potentiating functions.
Recently however, attempts have been made to moderate the
actual production of such proteins by interactions with
molecules that direct their synthesis, intracellular RNA.
25 By interfering with the production of proteins, it has been
hoped to effect therapeutic results with maximum effect and
minimal side effects. One approach for inhibiting specific

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gene expression is the use of oligonucleotide and
oligonucleotide analogs as antisense agents.

    Antisense methodology is the complementary
hybridization of relatively short oligonucleotides to
5 single-stranded mRNA or single-stranded DNA such that the
normal, essential functions of these intracellular nucleic
acids are disrupted. Hybridization is the sequence
specific hydrogen bonding of oligonucleotides to Watson-
Crick base pairs of RNA or single-stranded DNA. Such base
10 pairs are said to be complementary to one another.

    The naturally occurring event that provides the
disruption of the nucleic acid function, discussed by Cohen
in *Oligonucleotides: Antisense Inhibitors of Gene
Expression*, CRC Press, Inc., Boca Raton, FL (1989) is
15 thought to be of two types. The first, hybridization
arrest, denotes the terminating event in which the
oligonucleotide inhibitor binds to the target nucleic acid
and thus prevents, by simple steric hindrance, the binding
of essential proteins, most often ribosomes, to the nucleic
20 acid. Methyl phosphonate oligonucleotides; P.S. Miller &
P.O.P. Ts'O, *Anti-Cancer Drug Design*, 2:117-128 (1987), and
α-anomer oligonucleotides are the two most extensively
studied antisense agents which are thought to disrupt
nucleic acid function by hybridization arrest.

25   The second type of terminating event for
antisense oligonucleotides involves the enzymatic cleavage
of the targeted RNA by intracellular RNase H. The
oligonucleotide or oligonucleotide analog, which must be of
the deoxyribo type, hybridizes with the targeted RNA and
30 this duplex activates the RNase H enzyme to cleave the RNA
strand, thus destroying the normal function of the RNA.
Phosphorothioate oligonucleotides are the most prominent
example of an antisense agent which operates by this type
of antisense terminating event.

35   Considerable research is being directed to the
application of oligonucleotide and oligonucleotide analogs
as antisense agents for therapeutic purposes. All

applications of oligonucleotides as diagnostic, research
reagents, and potential therapeutic agents require that the
oligonucleotides or oligonucleotide analogs be synthesized
in large quantities, be transported across cell membranes

5   or taken up by cells, appropriately hybridize to targeted
RNA or DNA, and subsequently terminate or disrupt nucleic
acid function. These critical functions depend on the
initial stability of oligonucleotides toward nuclease
degradation.

10   A serious deficiency of oligonucleotides for
these purposes, particularly antisense therapeutics, is the
enzymatic degradation of the administered oligonucleotide
by a variety of ubiquitous nucleolytic enzymes,
intracellularly and extracellularly located, hereinafter

15 referred to as "nucleases". It is unlikely that
unmodified, "wild type", oligonucleotides will be useful
therapeutic agents because they are rapidly degraded by
nucleases. Modification of oligonucleotides to render them
resistant to nucleases is therefore currently a primary

20 focus of antisense research.

        Modifications of oligonucleotides to enhance
nuclease resistance have heretofore exclusively taken place
on the sugar-phosphate backbone, particularly on the
phosphorus atom. Phosphorothioates, methyl phosphonates,

25 phosphorimidates, and phosphorotriesters (phosphate
methylated DNA) have been reported to have various levels
of resistance to nucleases. However, while the ability of
an antisense oligonucleotide to bind to specific DNA or RNA
with fidelity is fundamental to antisense methodology,

30 modified phosphorous oligonucleotides, while providing
various degrees of nuclease resistance, suffer from
inferior hybridization properties.

        Due to the prochiral nature of the phosphorous
atom, modifications on the internal phosphorus atoms of

35 modified phosphorous oligonucleotides result in Rp and Sp
stereoisomers. Since a practical synthesis of stereo
regular oligonucleotides (all Rp or Sp phosphate linkages)

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is unknown, oligonucleotides with modified phosphorus atoms have n^2 isomers with n equal to the length or the number of the bases in the oligonucleotide. Furthermore, modifications on the phosphorus atom have unnatural bulk

5 about the phosphorodiester linkage which interferes with the conformation of the sugar-phosphate backbone and consequently, the stability of the duplex. The effects of phosphorus atom modifications cause inferior hybridization to the targeted nucleic acids relative to the unmodified

10 oligonucleotide hybridizing to the same target.

The relative ability of an oligonucleotide to bind to complementary nucleic acids is compared by determining the melting temperature of a particular hybridization complex. The melting temperature (T_m), a

15 characteristic physical property of double helixes, denotes the temperature in degrees centigrade at which 50% helical versus coil (unhybridized) forms are present. T_m is measured by using the UV spectrum to determine the formation and breakdown (melting) of hybridization. Base

20 stacking, which occurs during hybridization, is accompanied by a reduction in UV absorption (hypochromicity). Consequently a reduction in UV absorption indicates a higher T_m. The higher the T_m, the greater the strength of the binding of the strands. Non-Watson-Crick base pairing

25 has a strong destabilizing effect on the T_m. Consequently, absolute fidelity of base pairing is necessary to have optimal binding of an antisense oligonucleotide to its targeted RNA.

Considerable reduction in the hybridization

30 properties of methyl phosphonates and phosphorothioates has been reported by Cohen. Methyl phosphonates have a further disadvantage in that the duplex formed with RNA does not activate degradation by RNase H as an terminating event, but instead acts by hybridization arrest which can be

35 reversed due to a helical melting activity located on the ribosome. Phosphorothioates are highly resistant to most nucleases. However, phosphorothioates typically exhibit

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non-antisense modes of action, particularly the inhibition
of various enzyme functions due to nonspecific binding.
Enzyme inhibition by sequence-specific oligonucleotides
undermines the very basis of antisense chemotherapy.

5   Therefore, oligonucleotides modified to exhibit
resistance to nucleases, to activate the RNase H
terminating event, and to hybridize with appropriate
strength and fidelity to its targeted RNA (or DNA) are
greatly desired for antisense oligonucleotide therapeutics.

10   M. Ikehara et al., *European Journal of
Biochemistry* 139:447-450(1984) report the synthesis of a
mixed octamer containing one 2'-deoxy-2'-fluoroguanosine
residue or one 2'-deoxy-2'-fluoroadenine residue. W.
Guschlbauer and K. Jankowski, *Nucleic Acids Res.* 8:1421

15 (1980) have shown that the contribution of the N form
(3'-endo, 2'-exo) increases with the electronegativeness of
the 2'-substituent. Thus, 2'-deoxy-2'-fluorouridine
contains 85% of the C3'-endo conformer. M. Ikehara et al.,
Tetrahedron Letters 42:4073 (1979) have shown that a linear

20 relationship between the electronegativeness of
2'-substituents and the % N conformation (3'-endo-2'-exo)
of a series of 2'-deoxy- adenosines. M. Ikehara et al.,
Nucleic Acids Research 5:1877 (1978) have chemically
transformed 2'-deoxy-2'-fluoro- adenosine to its

25 5'-diphosphate. This was subsequently enzymatically
polymerized to provide poly(2'-deoxy-2'- fluoroadenylic
acid).

          Furthermore, evidence was presented which
indicates that 2'-substituted 2'-deoxyadenosines

30 polynucleotides resemble double stranded RNA rather than
DNA. M. Ikehara et al., *Nucleic Acids Res.* 5:3315 (1978)
show that a 2'-fluorine substituent in poly A, poly I, and
poly C duplexed to their U, C, or I complement are
significantly more stable than the ribo or deoxy poly

35 duplexes as determined by standard melting assays. M.
Ikehara et al., *Nucleic Acids Res.* 4:4249 (1978) show that
a 2'-chloro or bromo substituents in poly(2'-deoxy-

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adenylic acid) provides nuclease resistance. F. Eckstein et
al., *Biochemistry* 11:4336 (1972) show that
poly(2'-chloro-2'-deoxyuridylic acid) and
poly(2'-chloro-2'-deoxycytidylic acid) are resistant to
5   various nucleases. H. Inoue et al., *Nucleic Acids Research*
15:6131 (1987) describe the synthesis of mixed
oligonucleotide sequences containing 2'-OMe at every
nucleotide unit. The mixed 2'-OMe substituted sequences
hybridized to their ribooligonucleotide complement (RNA) as
10 strongly as the ribo-ribo duplex (RNA-RNA) which is
significantly stronger than the same sequence
ribo-deoxyribo heteroduplex (T_ms, 49.0 and 50.1 versus 33.0
degrees for nonamers). S. Shibahara et al., *Nucleic Acids
Research* 17:239 (1987) describe the synthesis of mixed
15 oligonucleotides sequences containing 2'-OMe at every
nucleotide unit. The mixed 2'-OMe substituted sequences
were designed to inhibit HIV replication.

It is thought that the composite of the hydroxyl
group's steric effect, its hydrogen bonding capabilities,
20 and its electronegativeness versus the properties of the
hydrogen atom is responsible for the gross structural
difference between RNA and DNA. Thermal melting studies
indicate that the order of duplex stability (hybridization)
of 2'-methoxy oligonucleotides is in the order of RNA-RNA,
25 RNA-DNA, DNA-DNA.

The 2'-deoxy-2'-halo, azido, amino, methoxy
homopolymers of several natural occurring nucleosides have
been prepared by polymerase processes. The required
2'-modified nucleosides monomers have not been incorporated
30 into oligonucleotides via nucleic acids synthesizer
machines. Thus, mixed sequence (sequence-specific)
oligonucleotides containing 2'-modifications at each sugar
are not known except for 2'-deoxy-2'-methoxy analogs.

OBJECTS OF THE INVENTION

It is a principal object of the invention to provide nuclease resistant, sugar modified oligonucleotides or oligonucleotide analogs for use in antisense

5   oligonucleotide diagnostics, research reagents, and therapeutics.

It is a further object of the invention to provide such oligonucleotides or oligonucleotides analogs which are effective in modulating the activity of a DNA or

10 an RNA.

Another object of the invention is to provide such oligonucleotides or oligonucleotide analogs which are less likely to invoke undesired or toxic side reactions.

Yet another object of the invention is to provide

15 research and diagnostic methods and materials for assaying bodily states in animals, especially diseased states.

A further object of the invention is to provide therapeutic and research methods and materials for the treatment of diseases through modulation of the activity of

20 DNA and RNA.

These and other objects will become apparent to persons of ordinary skill in the art from a review of the present specification and attendant claims.

SUMMARY OF THE INVENTION

25   In accordance with the present invention, compositions which are resistant to nuclease degradation and but which modulate the activity of DNA and RNA are provided. These compositions are comprised of sugar modified oligonucleotides or oligonucleotide analogs, the

30 targeting portions of which are specifically hybridizable with preselected nucleotide sequences of single-stranded or double-stranded DNA or RNA. The sugar modified oligonucleotides recognize and form double strands with single stranded DNA and RNA or triple strands with double

35 stranded DNA and RNA.

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The nuclease resistant oligonucleotides of this invention consist of a single strand of nucleic acid bases linked together through linking groups. The target portion of the nuclease resistant oligonucleotide may range in
5   length from about 5 to about 50 nucleic acid bases. However, in accordance with the preferred embodiment of this invention, a target sequence of about 15 bases in length is optimal.

The nucleic acid bases may be pyrimidines such as
10 thymine, uracil or cytosine, or purines such as guanine or adenine, or both, arranged in a specific sequence. The sugar moiety of such bases may be of the deoxyribose or ribose type. The groups linking the bases together may be the usual sugar phosphate nucleic acid backbone, but may
15 also be modified as a phosphorothioate, methylphosphonate, or phosphate alkylated moiety to further enhance the sugar modified oligonucleotide properties, along with removal of a 5'-methylene group and/or carbocyclic sugar.

In accordance with this invention, the targeting
20 portion is an analog of an oligonucleotide wherein at least one of the 2'-deoxy ribofuranosyl moieties of the nucleoside unit is modified. A hydrogen or a hydroxyl, halo, azido, amino, methoxy or alkyl group may be added. For example, H, OH, F, CN, CF_3, OCF_3, OCN, O-alkyl, S-alkyl,
25 SOMe, SO_2Me, ONO_2, NO_2, N_3, NH_2, NH-alkyl, $OCH_2CH=CH_2$ (allyloxy), $OCH=CH_2$, OCCH where alkyl is a straight or branched chain of C1 to C12 may be used, with unsaturation within the carbon chain, such as allyloxy being particularly preferred.
30   The resulting novel oligonucleotides or oligonucleotide analogs are resistant to nuclease degradation and exhibit hybridization properties of higher quality relative to wild type (DNA-DNA and RNA-DNA) duplexes and the phosphorus modified oligonucleotide
35 antisense duplexes containing phosphorothioates, methylphosphonates, phophoramidates and phosphorotriesters.

The invention is also directed to methods for modulating the production of a protein by an organism comprising contacting the organism with a composition formulated in accordance with the foregoing considerations.

5   It is preferred that the RNA or DNA portion which is to be modulated be preselected to comprise that portion of DNA or RNA which codes for the protein whose formation is to be modulated. The targeting portion of the composition to be employed is, thus, selected to be complementary to the

10 preselected portion of DNA or RNA, that is to be an antisense oligonucleotide for that portion.

This invention is also directed to methods of treating an organism having a disease characterized by the undesired production of a protein. This method comprises

15 contacting the organism with a composition in accordance with the foregoing considerations. The composition is preferably one which is designed to specifically bind with messenger RNA which codes for the protein whose production is to be inhibited.

20 The invention further is directed to diagnostic methods for detecting the presence or absence of abnormal RNA molecules or abnormal or inappropriate expression of normal RNA molecules in organisms or cells.

The invention is also directed to methods for the

25 selective binding of RNA for research and diagnostic purposes. Such selective, strong binding is accomplished by interacting such RNA or DNA with compositions of the invention which are resistant to degradative nucleases and hybridize stronger and with greater fidelity than any other

30 known oligonucleotide or oligonucleotide analog.

Additionally this invention is directed to a method of synthesis of 2'-deoxy-2'-substituted nucleosides, particularly guanosine compounds. In accordance with this method, the 2'-hydroxyl moiety of guanosine is first

35 oxidized and then reduced with inversion about the 2' position to yield 9-(β-$\underline{D}$-arabinofuranosyl)guanine. The 2' arabino hydroxyl group is derivatized with a leaving group.

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Nucleophilic displacement of the leaving group with a
nucleophile is accomplished with a further inversion to
give the 2'-deoxy-2'-substituted guanosine compound.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

5   The compositions useful for modulating the
activity of an RNA or DNA molecule in accordance with this
invention generally comprise a sugar modified
oligonucleotide containing a target sequence which is
specifically hybridizable with a preselected nucleotide
10   sequence of single stranded or double stranded DNA or RNA
molecule and which is nuclease resistant.

          It is generally desirable to select a sequence of
DNA or RNA for or which is involved in the production of
proteins whose synthesis is ultimately to be modulated or
15   inhibited in entirety. The targeting portion of the
composition is generally an oligonucleotide analog. It is
synthesized, conveniently through solid state synthesis of
known methodology, to be complementary to or at least to be
specifically hybridizable with the preselected nucleotide
20   sequence of the RNA or DNA. Nucleic acid synthesizers are
commercially available and their use is generally
understood by persons of ordinary skill in the art as being
effective in generating nearly any oligonucleotide of
reasonable length which may be desired.

25   In the context of this invention, the term
"oligonucleotide" refers to a plurality of joined
nucleotide units formed in a specific sequence from
naturally occurring bases and pentofuranosyl groups joined
through a sugar group by native phosphodiester bonds.
30   These nucleotide units may be nucleic acid bases such as
guanine, adenine, cytosine, thymine or uracil. The sugar
group may be deoxyribose or ribose. This term refers to
both naturally occurring or synthetic species formed from
naturally occurring subunits.

35   "Oligonucleotide analog" as the term is used in
connection with this invention, refers to moieties which

function similarly to oligonucleotides but which have non-
naturally occurring portions. Oligonucleotide analogs may
have altered sugar moieties or inter-sugar linkages, for
example, phosphorothioates and other sulfur containing

5   species which are known for use in the art.
Oligonucleotide analogs may also comprise altered base
units or other modifications consistent with the spirit of
this invention, and in particular such modifications as may
increase nuclease resistance of the oligonucleotide

10 composition in order to facilitate antisense therapeutic,
diagnostic or research reagent use of a particular
oligonucleotide.
        It is generally preferred for use in some
embodiments of this invention that some positions of the

15 nucleotide base be substituted in order to increase the
nuclease resistance of the composition while maintaining
the integrity of the oligonucleotide binding capabilities.
        It is preferred in some embodiments of the
present invention to employ further modified

20 oligonucleotides. In this context, modified
oligonucleotide analogs refers to a structure which is
generally similar to native oligonucleotides, but which
have been modified in one or more significant ways.
        Such modifications may take place at the sugar

25 backbone of the invention. It is generally preferred to
enhance the ability of the target sequence of the sugar
modified oligonucleotides to penetrate into the
intracellular spaces of cells where the messenger RNA or
DNA, which are the targets of the overall composition,

30 reside. Therefore, it is generally preferred to provide
modifications of oligonucleotides which are substantially
less ionic than native forms in order to facilitate
penetration of the oligonucleotide into the intracellular
spaces. Any of the existing or yet to be discovered

35 methods for accomplishing this goal may be employed in
accordance with the practice of the present invention. At
present, it has been found preferable to employ

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substitutions for the phosphorodiester bond, which
substitutions are not only relatively less ionic than the
naturally occurring bonds but are also substantially non-
chiral.

5   As will be appreciated, the phosphorus atom in
the phosphorodiester linkage is "pro-chiral".
Modifications at the phosphorus, such as is done in methyl
phosphonates and phosphorothioates type oligonucleotides,
results in essentially chiral structures. Chirality
10   results in the existence of two isomers at each chiral
center which may interact differently with cellular
molecules. Such an unresolved mixture of isomers may
inhibit the transport of the resulting compositions into
the intracellular spaces or decrease the affinity and
15   specificity of hybridization to the specific target RNA or
DNA. Thus, it is preferred in some embodiments of this
invention to employ substantially non-ionic, substantially
non-chiral entities in lieu of some or all of the
phosphorodiester bonds. For this purpose, short chain
20   alkyl or cycloalkyl structures especially C_2-C_4 structures
are preferred. As is set forth in an application filed on
even date herewith and assigned to a common assignee
hereof, said application being entitled "Polyamine
Oligonucleotides to Enhance Cellular Uptake," application
25   serial number 558,663 filed July 27, 1990 (attorney docket
ISIS-24) the modification of the sugar structure including
the elimination of one of the oxygen functionality may
permit the introduction of such substantially non-chiral,
non-ionic substituents in this position. The entirety of
30   the disclosure of application serial number 558,663 is
incorporation herein by reference in order to disclose more
fully such modifications.

        In keeping with the goals of the invention are
the standard backbone modifications such as substituting P
35   for S, Me-P, MeO-P, H_2N-P, etc. These substitutions are
thought in some cases to enhance the sugar modified
oligonucleotide properties.

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The targeting portion of the compositions of the present invention, are preferably oligonucleotide analogs having 5 to about 50 base units. It is more preferred that such functionalities have from 8 to about 40 base units and
5   even more preferred that from about 12 to 20 base units be employed. Oligonucleotides or oligonucleotide analogs having about 15 base units are preferable for the practice of certain embodiments of the present invention.

It is desired that the targeting portion be
10 adapted so as to be specifically hybridizable with the preselected nucleotide sequence of the RNA or DNA selected for modulation. Oligonucleotide analogs particularly suited for the practice of one or more embodiments of the present invention comprise 2'-sugar modified
15 oligonucleotides wherein one or more of the 2'-deoxy ribofuranosyl moieties of the nucleoside unit is modified with a hydrogen or hydroxyl, halo, azido, amino, alkyoxy, thioalkoxy, alkylamino or alkyl group. For example, the substitutions which may occur include H, OH, F, CN, CF_3,
20 OCF_3, OCN, O-alkyl, S-alkyl, SOMe, SO_2Me, ONO_2, NO_2, N_3, NH_2, NH-alkyl, $OCH=CH_2$, OCCH where alkyl is a straight or branched chain of C_1 to C_{12} with unsaturation within the carbon chain such as allyloxy.

These modified bases are linked together and to
25 the rest of the oligonucleotide or oligonucleotide analog through a sugar linking group. The linking group may be any of those structures described herein which are capable of linking sugar moieties of oligonucleotides together to form the targeting portion of the compositions of this
30 invention. It is preferred that these sugar linking groups comprise the phosphodiester structure or a derivative of such. Derivatives of the phosphodiester structure may include substitution of a sulphur, an alkoxy group such as methyl, methyl oxide, or amine group for an oxygen. The
35 sugar phosphate nucleic acid backbone may be modified as a phosphorothioate, alkylphosphonate such as methylphosphonate or phosphate alkylated moiety (a

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phosphotriester). The phosphodiester linkage may also be
replaced by a carbon or ether linkage.

In further embodiment of this invention, a
linking moiety has been devised to allow the direct
5 attachment of a modified unit to the terminal position of
the 3'-end of the modified oligonucleotides. Thus, an
ester, or more preferably a bromomethylketo group, is
attached to the 3'-hydroxyl of a modified 2'-modified
nucleoside having its 5'-hydroxyl protected with a
10 dimethoxytriphenylmethyl group and, if the heterocycle is
of the cytosine series, having that heterocycle protected
with a benzoyl protecting group. If the required targeting
sequence has a terminal 3'-thymine or cytosine base, the
desired modified thymine or cytosine base containing the
15 bromomethylketo linker is utilized as the first monomer to
attach to the control pore glass (CPG) solid support which
contains a normal nucleoside attached via its 3'-hydroxyl
group. The base sensitive ester linkage attaching the 2'-
modified nucleoside to the nucleoside attached to the CPG
20 is cleaved under the usual concentrated ammonium hydroxide
conditions that are utilized to remove the oligonucleotide
from the CPG support. This will allow the modified
oligonucleotide to have a 2'-modified unit at its terminal,
3'-end.
25   Cleavage of oligonucleotides by nucleolytic
enzymes require the formation of an enzyme-substrate
complex, or in particular a nuclease-oligonucleotide
complex. The nuclease enzymes will generally require
specific binding sites located on the oligonucleotides for
30 appropriate attachment. If the oligonucleotide binding
sites are removed or hindered such that the nucleases will
not attach to the oligonucleotides, the nuclease resistant
oligonucleotides result. In the case of restriction
endonucleases that cleave sequence-specific palindromic
35 double-stranded DNA, certain binding sites such as the ring
nitrogen in the 3- and 7-positions have been identified as
required biding sites. Removal of one or more of these

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sites or hindering the nuclease approach to these
particular positions within the recognition sequence has
provided various levels or resistance to the specific
nucleases.

5   This invention provides antisense
oligonucleotides characterized by superior hybridizing
properties. We have discovered from structure activity
relationships studies that a significant increase in
binding (T_m)s of certain 2'-sugar modified oligonucleotides
10 to its RNA target (complement) is correlated with an
increased "A" type conformation of the heteroduplex.
Furthermore, absolute fidelity of the modified
oligonucleotides is maintained. The increased binding of
our 2'-sugar modified sequence-specific oligonucleotides
15 provides superior potency and specificity compared to
phosphorus modified antisense oligonucleotides such as
methyl phosphonates, phosphorothioates, phosphate triesters
and phosphoramidites as known in the literature.

        The only structural difference between DNA and
20 RNA duplexes is an hydrogen atom in the 2'-position of the
DNA ribofuranosyl moieties versus a hydroxyl group in the
2'-position of the RNA ribofuranosyl moieties (assuming
that the presence or absence of a methyl group in the
uracil ring system has no effect). However, gross
25 conformational differences exist between DNA and RNA
duplexes.

        It is known from X-ray diffraction analysis of
nucleic acid fibers, Arnott and Hukins, *Biochemical and
Biophysical Research Communication*, 47:1504-1510 (1970),
30 and analysis of crystals of double-stranded nucleic acids
that DNA takes a "B" form structure and that RNA only takes
the much more rigid "A" form structure. The difference
between the sugar puckering (C2' endo for "B" form DNA and
C3' endo for A-form RNA) of the nucleoside monomeric units
35 of DNA and RNA is the major conformational difference
between double-stranded nucleic acids.

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The primary contributor to the pentofuranosyl moiety conformation is the nature of the substituent in the 2'-position. Thus, the population of the C3'-endo form increases with respect to the C2'-endo as the

5 electronegativity of the 2'-substituent increases. For example, among 2'-deoxy-2'-halo-adenine nucleosides, the 2'-fluoro derivative exhibits the largest population (65%) of C3'-endo, and the 2'-iodo shows the lowest (7%). Those of the adenosine (2'-OH) and deoxyadenosine (2'-H) are 36%

10 and 19%, respectively. Furthermore, the effect of the 2'-fluoro group of adenine dinucleotides (2'-deoxy-2'-fluoroadenosine - 2'-deoxy-2'- fluoroadenosine or uridine) is further correlated to the stabilization of the stacked conformations more than ribo or deoxyribo

15 modified dimers. Research indicates that the dinucleosides phosphates have a stacked conformation with a geometry similar to that of A-A but with a greater extent of base-base overlapping than A-A. It was assumed that the highly polar nature of the C2'-F bond and the extreme

20 preference for C3'-endo puckering may stabilize the stacked conformation in an "A" structure.

Data from UV hypochromicity, circular dichromism, and 'H NMR also indicate that the degree of stacking decreases as the electronegativeness of halogen decreases.

25 Furthermore, a steric bulkiness in the 2'-position is better accommodated in an "A" form duplex than a "B" form duplex.

Thus, a 2'-substituent on the 3'-nucleotidyl unit of a dinucleoside monophosphate is thought to exert a

30 number of effects on the stacking conformation: steric repulsion, furanose puckering preference, electrostatic repulsion, hydrophobic attraction, and hydrogen bonding capabilities. These substituent effects are thought to be determined by the molecular size, electronegativity, and

35 hydrophobicity of the substituent.

The 2'-iodo substituted nucleosides possess the lowest C3'-endo population (7%) of the halogen series.

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Thus, on steric effects alone, one would predict an
2'-iodo or similar groups would contribute stacking
destabilizing properties and thus reduced binding (T_m)s for
antisense oligonucleotides. However, the lower
5 electronegativeness and high hydrophobic attractive forces
of the iodine atom and similar groups complicates the
ability to predict stacking stabilities and binding
strengths.

        Studies with the 2'-OMe modification of 2'-deoxy
10 guanosine, cytidine, and uridine dinucleoside phosphates
exhibit enhanced stacking effects with respect to the
corresponding unmethylated species (2'-OH). In this case,
the hydrophobic attractive forces of the methyl group tend
to overcome the destablilizing effects of its steric
15 bulkiness (hindrance).

        2'-Fluoro-2'-deoxyadenosine has been determined
to have an unusually high population of 3'-endo puckering
among nucleosides. Adenosine, 2'-deoxyadenosine, and other
derivatives typically have population below 40% in the
20 3'-endo conformer. It is known that a nucleoside residue in
well-stacked oligonucleotides favors 3'-endo ribofuranose
puckering.

        Melting temperatures (complementary binding) are
increased with the 2'-substituted adenosine diphosphates.
25 It is not clear whether the 3'-endo preference of the
conformation or the presence of the substituent is
responsible for the increased binding. However, as noted,
greater overlap of adjacent bases (stacking) can be
achieved with the 3'-endo conformations.

30   The present novel approach to obtaining stronger
binding is to prepare antisense RNA mimics to bind to the
targeted RNA. Therefore, a random structure-activity
relationship approach was undertaken to discover nuclease
resistant antisense oligonucleotides that maintained
35 appropriate hybridization properties.

        A series of 2'-deoxy-2'-modified nucleosides of
adenine, guanine, cytosine, thymidine and certain analogs

of these bases have been prepared and have been inserted as
the modified nucleosides into sequence-specific
oligonucleotides via solid phase nucleic acid synthesis.
The novel antisense oligonucleotides were assayed for their
5   ability to resist degradation by nucleases and to possess
hybridization properties comparable to the unmodified
parent oligonucleotide. Initially, small electronegative
atoms or groups were selected because these type are not
likely to sterically interfere with required Watson-Crick
10   base pair hydrogen bonding (hybridization). However,
electronic changes due to the electronegativeness of the
atom or group in the 2'-position may profoundly effect the
sugar conformation. During our structure activity
relationship studies we discovered that the sugar modified
15   oligonucleotides hybridized to the targeted RNA stronger
than the unmodified (2'-deoxyribosyl type).
        2'-Substituted oligonucleotides are synthesized
by the standard solid phase, automated nucleic acid
synthesizer such as the Applied Biosystems, Incorporated
20   380B or MilliGen/ Biosearch 7500 or 8800. Triester,
phosphoramidite, or hydrogen phosphonate coupling
chemistries (Oligonucleotides. Antisense Inhibitors of Gene
Expression. M. Caruthers, pp 7-24, Edited by J. S. Cohen,
CRC Press, Inc. Boca Raton, Florida, 1989) are used in with
25   these synthesizers to provide the desired oligonucleotides.
The Beaucage reagent (Journal of American Chemical Society,
112, 1253-1255, 1990) or elemental sulfur (S. Beaucage et
al., Tetrahedron Letters, 22,1859-1862, 1981) is used with
phosphoramidite or hydrogen phosphonate chemistries to
30   provide 2'-substituted phosphorothioate oligonucleotides.
        The requisite 2'-substituted nucleosides (A, G,
C, T(U), and nucleic acid base analogs) are generally
prepared by modification of several literature procedures
as described below.
35   **Procedure 1.** Nucleophilic Displacement of
2'-Leaving Group in Arabino Purine Nucleosides.
Nucleophilic displacement of a leaving group in the 2'-up

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position (2'-deoxy-2'-(leaving group)arabino sugar) of
adenine or guanine or their analog nucleosides. General
synthetic procedures of this type have been described by M.
Ikehara et al., *Tetrahedron* 34:1133-1138 (1978); ibid.,
5   31:1369-1372 (1975); *Chemistry and Pharmaceutical Bulletin,*
26:2449-2453 (1978); ibid., 26:240-244 (1978); M. Ikehara
Accounts of Chemical Research, 2:47-53 (1969); and R.
Ranganathan *Tetrahedron Letters,* 15:1291-1294 (1977).
        Procedure 2. Nucleophilic Displacement of
10 2,2'-Anhydro Pyrimidines. Nucleosides thymine, uracil,
cytosine or their analogs are converted to 2'-substituted
nucleosides by the intermediacy of 2,2'-cycloanhydro
nucleoside as described by J.J. Fox, et al., *Journal of
Organic Chemistry,* 29:558-564 (1964).
15   **Procedure 3.** 2'-Coupling Reactions.
Appropriately 3',5'-sugar and base protected purine and
pyrimidine nucleosides having a unprotected 2'-hydroxyl
group are coupled with electrophilic reagents such as
methyl iodide and diazomethane to provide the mixed
20 sequences containing a 2'-OMe group H. Inoue, et al.,
Nucleic Acids Research 15: 6131-6148.
        Procedure 4. 2-Deoxy-2-substituted Ribosylations.
2-Substituted-2-deoxyribosylation of the appropriately
protected nucleic acid bases and nucleic acids base analogs
25 has been reported by E. T. Jarvi, et al., *Nucleosides &
Nucleotides* 8:1111-1114 (1989) and L. W. Hertel, et al.,
Journal of Organic Chemistry 53:2406-2409 (1988).
        Procedure 5. Enzymatic Synthesis of 2'-Deoxy-2'-
Substituted Nucleosides. The 2-Deoxy-2-substituted
30 glycosyl transfer from one nucleoside to another with the
aid of pyrimidine and purine ribo or deoxyribo
phosphorolyses has been described by J. R. Rideout and T.
A. Krenitsky, United States Patent # 4,381,344 (1983).
        Procedure 6. Conversion of 2'-Substituents Into
35 New Substituents. 2'-Substituted-2'-deoxynucleosides are
converted into new substituents via standard chemical
manipulations. For example, S. Chladek et al., *Journal of*

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Carbohydrates, Nucleosides & Nucleotides 7:63-75 (1980)
describes the conversion of 2'-deoxy-2'-azidoadenosine,
prepared from arabinofuranosyladenine, into 2'-deoxy-2'-
aminoadenosine.

5   **Procedure 7. Free Radical Reactions.** Conversions
of halogen substituted nucleosides into 2'-deoxy-2'-
substituted nucleosides via free radical reactions has been
described by K. E. B. Parkes and K. Taylor, *Tetrahedron
Letters* 29:2995-2996 (1988).

10   **Procedure 8.** Conversion of Ribonucleosides to
2'-Deoxy-2'-Substituted Nucleoside. Appropriately
3',5'-sugar and base protected purine and pyrimidine
nucleosides having a unprotected 2'-hydroxyl group are
converted to 2'-deoxy-2'- substituted nucleosides by the
15 process of oxidation to the 2'-keto group, reaction with
nucleophilic reagents, and finally 2'-deoxygenation.
Procedures of this type have been described by F. De las
Heras, et al., *Tetrahedron Letters* 29:941-944 (1988).
            Procedure 9. In a preferred process of the
20 invention, 2'-deoxy-Substituted guanosine compounds are
prepared via n (arabinofuranosyl)guanine intermediate
obtained via an oxidation-reduction reaction. A leaving
group at the 2' position of the arabinofuranosyl sugar
moiety of the intermediate arabino compound is displaced
25 via an SN_2 reaction with an appropriate nucleophile. This
procedure thus incorporate principles of both Procedure 1
and Procedure 8 above. 2'-Deoxy-2'-fluoroguanosine is
preferably prepared via this procedure. The intermediate
arabino compound was obtained utilizing a variation of the
30 oxidation-reduction procedure of Hansske, F., Madej, D. and
Robins, M.J. (1984), *Tetrahedron*, 40:125. According to
this invention, the reduction was effected starting at -
78°C and allowing the reduction reaction to exothermically
warm to about -2°C. This results in a high yield of the
35 intermediate arabino compound.
            In conjunction with use of a low temperature
reduction, utilization of a tetraisopropyldisiloxane

blocking group (a "TPDS" group) for the 3' and 5' positions
of the starting guanosine compound contributes in an
improved ratio of intermediate arabino compound verses the
ribo compound following oxidization and reduction.

5   Following oxidation/reduction, the N^2 guanine amino nitrogen
and the 2'-hydroxyl moieties of the intermediate arabino
compound are blocked with isobutyryl protecting groups
("Ibu" groups). The tetraisopropyldisiloxane blocking
group is removed and the 3' and 5' hydroxyl's are further

10   protected with a second blocking group, a tetrahydropyranyl
blocking group (a "THP" group). The isobutyryl group is
selectively removed from 2'-hydroxyl group followed by
derivation of the 2' position with a triflate (a
trifluoromethylsulfonyl) leaving group. The triflate

15   moiety was then displaced with inversion about the 2'
position to yield the desire 2'-deoxy-2'-fluoroguanosine
compound.

        In addition to the triflate leaving group, other
leaving groups include but are not necessarily limited to

20   alkysulfonyl, substituted alkylsulfonyl, arylsulfonyl,
substituted arylsulfonyl, heterocyclosulfonyl or
trichloroacetimidate. Representative examples include p-
(2,4-dinitroanilino)benzenesulfonyl, benzenesulfonyl,
methylsulfonyl, p-methylbenzenesulfonyl, p-bromo-

25   benzenesulfonyl, trichloroacetimidate, acyloxy, 2,2,2-
trifluoro-ethanesulfonyl, imidazolesulfonyl and 2,4,6-
trichlorophenyl.

        The isobutyryl group remaining on the N^2
heterocyclic amino moiety of the guanine ring can be

30   removed to yield a completely deblocked nucleoside;
however, preferably for incorporation of the 2'-deoxy-2'-
substituted compound in an oligonucleotide, deblocking of
the N^2 isobutyryl protecting group is deferred until after
oligonucleotide synthesis is complete. Normally for use on

35   automated nucleic acid synthesizers, blocking of the N^2
guanine amino moiety with an isobutyryl group is preferred.
Thus advantageously, the N^2-isobutyryl blocked 2'-deoxy-2'-

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substituted guanosine compounds resulting from the method
of the invention can be directly used for oligonucleotide
synthesis on automatic nucleic acid synthesizers.

The oligonucleotides or oligonucleotide analogs
5 of this invention can be used in diagnostics, therapeutics,
and as research reagents and kits. For therapeutic use the
oligonucleotide is administered to an animal suffering from
a disease affected by some protein. It is preferred to
administer to patients suspected of suffering from such a
10 disease with amounts of oligonucleotide which are effective
to reduce the symptemology of that disease. It is within
the scope of a person's skill in the art to determine
optimum dosages and treatment schedules for such treatment
regimens.

15   It is generally preferred to apply the
therapeutic agents in accordance with this invention
internally such as orally, intravenously, or
intramuscularly. Other forms of administration, such as
transdermally, topically, or intralesionally may also be
20 useful. Inclusion in suppositories may also be useful.
Use of pharmacologically acceptable carriers is also
preferred for some embodiments.

The following examples illustrate the practice of
this invention.

25 **EXAMPLE 1 - Preparation of 2'-Deoxy-2'-fluoro Modified
Oligonucleotides.**

        **A. N^6-Benzoyl-[2'-deoxy-2'-fluoro-5'-O-(4,4'-di-
        methoxytrityl)]adenosine-3'-O-(N,N-diisopropyl
        -β-cyanoethyl phosphoramidite.**

30   N^6-Benzoyl-9-(2'-fluoro-β-D-ribofuranosyl)adenine
was prepared from 9-β-D-arabinofuranosyladenine in a
five-step synthesis using a modification of a procedure
reported by M. Ikehara at al., *Nucleosides and Nucleotides*
2:373-385 (1983). Thus, the N^6-benzoyl derivative was
35 obtained in good yield utilizing the method of transient
protection with chlorotrimethylsilane. R.A.Jones, *J. Am.*

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Chem. Soc. 104:1316 (1982). Selective protection of the 3'
and 5'-hydroxyl groups of N^6-Benzoyl-9-β-D-arabino-
furanosyladenine with tetrahydropyranyl (THP) was
accomplished by modification of a literature procedure G.

5　Butke, et al., in *Nucleic Acid Chemistry*, Part 3:149-152,
Townsend, L.B. and Tipson, R.S. eds., (J. Wiley and Sons,
New York 1986) to yield N^6-Benzoyl-9-[3',5'-di-O-
(tetrahydropyran-2-yl)-β-D-arabino furanosyl]adenine in
good yield.　Treatment of N^6-Benzoyl-9-

10　[3',5'-di-O-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]
adenine with trifluoromethanesulfonic anhydride in
dichloromethane gave the 2'-triflate derivative
N^6-Benzoyl-9-[2'-O-trifluoromethylsulfonyl-3',5'-di-O-
tetrahydropyran-2-yl)-β-D-arabino furanosyl]adenine which

15　was not isolated due to its lability.　Displacement of the
2'-triflate group was effected by reaction with tetra-
butylammonium fluoride in tetrahydrofuran to obtain a
moderate yield of the 2'-fluoro derivative N^6-Benzoyl-9-[2'
-fluoro-3',5'-di-O-tetrahydro-pyran-2-yl)-β-D-

20　arabinofuranosyl]adenine.　Deprotection of the THP groups
of N^6-Benzoyl-9-[2'-fluoro-3',5'-di-O-(tetrahydropyran-2-yl)
-β-D-arabino furanosyl]adenine was accomplished by
treatment with Dowex-50W in methanol to yield N^6-benzoyl-9-
(2'-deoxy-2'-fluoro-β-D-ribofuranosyl)adenine in moderate

25　yield.　The ^{1}H-NMR spectrum of 6 was in agreement with the
literature values. M. Ikehara and H. Miki, *Chem. Pharm.*
Bull. 26: 2449-2453 (1978).　Standard methodologies were
employed to obtain the 5'-dimethoxytrityl-3'-
phosphoramidite intermediates N^6-Benzoyl-9-[2'-fluoro-5'-O-

30　(4,4'-dimethoxytrityl)- β-D-ribofuranosyl]adenine and
N^6-Benzoyl-[2'-deoxy-2'-fluoro-5'-O-(4,4'-dimethoxytrityl)]
adenosine-3'-O-(N,N-diisopropyl-β-cyanoethyl-
phosphoramidite. K. K. Ogilvie, *Can J. Chem.* 67: 831-839
(1989).

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B.   N⁶-Benzoyl-9-β-D-arabinofuranosyladenine.

9-β-D-arabinofuranosyladenine (1.07 g, 4.00 m.mol) was dissolved in anhydrous pyridine (20 mL) and anhydrous dimethylformamide (20 mℓ) under an argon

5   atmosphere. The solution was cooled to ice temperature and chlorotrimethylsilane (3.88 mℓ, 30.6 m.mol) was added slowly to the reaction mixture via syringe. After stirring the reaction mixture at ice temperature for 30 minutes, benzoyl chloride (2.32 mℓ, 20 m.mol) was added slowly. The

10 reaction mixture was allowed to warm to 20°C and stirred for 2 hours. After cooling the reaction mixture to ice temperature, cold water (8 mℓ) was added and the mixture was stirred for 15 minutes. Concentrated ammonium hydroxide (8 mℓ) was slowly added to the reaction mixture

15 to give a final concentration of 2M of ammonia. After stirring the cold reaction mixture for 30 minutes, the solvent was evaporated in vacuo (60 torr) at 20° C followed by evaporation in vacuo (1 torr) at 40° C to give an oil. This oil was triturated with diethyl ether (50 mℓ) to give

20 a solid which was filtered and washed with diethyl ether three times. This crude solid was triturated in methanol (100 mℓ) at reflux temperature three times and the solvent was evaporated to yield N⁶-benzoyl-9-(β-D-arabino-furanosyladenine)adenine as a solid (1.50 g, 100%).

25   **C. N⁶-Benzoyl-9-[3",5"-di-)-tetrahydropyran-2-**
             yl)-D-arabinofuranosyl] adenine.

N⁶-benzoyl-9-(β-D-arabinofuranosyl)adenine (2.62 g, 7.06 m.mol) was dissolved in anhydrous dimethylformamide (150 mℓ) under an argon atmosphere and p-toluenesulfonic

30 acid monohydrate (1.32 g, 6.92 m.mol) was added. This solution was cooled to ice temperature and dihydropyran (1.26 mℓ, 13.8 m.mol) was added via syringe. The reaction mixture was allowed to warm to 20° C. Over a period of 5 hours a total of 10 equivalents of dihydropyran were added

35 in 2 equivalent amounts in the fashion described. The reaction mixture was cooled to ice temperature and

saturated aqueous sodium bicarbonate was added slowly to a
pH of 8, then water was added to a volume of 750 mℓ. The
aqueous mixture was extracted with methylene chloride four
times (4 x 200 mℓ), and the organic phases were combined

5   and dried over magnesium sulfate. The solids were filtered
and the solvent was evaporated in vacuo (60 torr) at 30° C
to give a small volume of liquid which was evaporated in
vacuo (1 torr) at 40° C to give an oil. This oil was
coevaporated with p-xylene in vacuo at 40° to give an oil

10 which was dissolved in methylene chloride (100 mℓ). Hexane
(200 mℓ) was added to the solution and the lower-boiling
solvent was evaporated in vacuo at 30° C to leave a white
solid suspended in hexane. This solid was filtered and
washed with hexane three times (3 x 10 mℓ) then purified by

15 column chromatography using silica and methylene
chloride-methanol (93:7, v/v) as eluent. The first
fraction yielded the title compound 3 as a white foam (3.19
g, 83 %) and a second fraction gave a white foam (0.81 g)
which was characterized as the 5'-mono-tetrahydropyranyl

20 derivative of N^6-benzoyl-9-(β-D-arabinofuranosyl)adenine.

D. N^6-Benzoyl-9-[2'-O-trifluoromethylsulfonyl-
3',5'-di-O-(tetrahydropyran-2-yl)-β-D-
arabinofuranosyl]adenine.

N^6-Benzoyl-9-[3',5'-di-O-(tetrahydropyran-2-yl)-

25 β-D-arabinofuranosyl]adenine (2.65 g, 4.91 m.mol) was
dissolved in anhydrous pyridine (20 mℓ) and the solvent was
evaporated in vacuo (1 mm Hg) at 40° C. The resulting oil
was dissolved in anhydrous methylene chloride (130 mℓ)
under an argon atmosphere and anhydrous pyridine (3.34 ml,

30 41.3 m.mol) and N,N-dimethylaminopyridine (1.95 g, 16.0
mmol) were added. The reaction mixture was cooled to ice
temperature and trifluoromethanesulfonic anhydride (1.36
ml, 8.05 mmol) was added slowly via syringe. After
stirring the reaction mixture at ice temperature for 1 h,

35 it was poured into cold saturated aqueous sodium
bicarbonate (140 mℓ). The mixture was shaken and the

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organic phase was separated and kept at ice temperature.
The aqueous phase was extracted with methylene chloride two
more times (2 x 140 mℓ). The organic extracts which were
diligently kept cold were combined and dried over magnesium
5   sulfate. The solvent was evaporated in vacuo (60 torr) at
20° C then evaporated in vacuo (1 torr) at 20° C to give
N^6-Benzoyl-9-[2'-O-trifluoromethylsulfonyl-3',5'
-di-O-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]adenine
as a crude oil which was not purified further.

10   E.   N^6-Benzoyl-9-[2'-fluoro-3',5'-di-O-
                  (tetrahydropyran-2-yl)-β-D-arabino-
                  furanosyl]adenine.
                  N^6-Benzoyl-9-[2'-O-trifluoromethylsulfonyl-3',5'
-di-O-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]adenine
15 (<4.9 mmol) as a crude oil was dissolved in anhydrous
tetrahydro-furan (120 mℓ) and this solution was cooled to
ice temperature under an argon atmosphere.
Tetrabutylammonium fluoride as the hydrate (12.8 g, 49.1
mmol) was dissolved in anhydrous tetrahydrofuran (50 mℓ)
20 and half of this volume was slowly added via syringe to the
cold reaction mixture. After stirring at ice temperature
for 1 hour, the remainder of the reagent was added slowly.
The reaction mixture was stirred at ice temperature for an
additional 1 hour, then the solvent was evaporated in vacuo
25 (60 torr) at 20° C to give an oil. This oil was dissolved in
methylene chloride (250 mℓ) and washed with brine three
times. The organic phase was separated and dried over
magnesium sulfate. The solids were filtered and the
solvent was evaporated to give an oil. The crude product
30 was purified by column chromatography using silica in a
sintered-glass funnel (600 mℓ) and ethyl acetate was used
as eluent. N^6-Benzoyl-9-[2'-deoxy-2'-fluoro-3',5'-di-O-
(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]adenine was
obtained as an oil (2.03 g, 76%).

F. N^6-Benzoyl-9-(2'-deoxy-2'-fluoro-
      β-D-ribofuranosyl)adenine.

      N^6-Benzoyl-9-[2'-fluoro-3',5'-di-O-
tetrahydropyran-2-yl)-β-D-arabinofuranosyl]adenine (1.31 g,

5   2.42 mmol) was dissolved in methanol (60 mℓ), and Dowex 50W
      x 2-100 (4 cm3, 2.4 m.eq) was added to the reaction
      mixture. The reaction mixture was stirred at 20° C for 1
      hour then cooled to ice temperature. Triethylamine (5 mℓ)
      was then slowly added to the cold reaction mixture to a pH

10   of 12. The resin was filtered and washed with 30%
      triethylamine in methanol until the wash no longer
      contained UV absorbing material. Toluene (50 mℓ) was added
      to the washes and the solvent was evaporated at 24° C in
      vacuo (60 torr then 1 torr) to give a residue. This

15   residue was partially dissolved in methylene chloride (30
      mℓ) and the solvent was transferred to a separatory funnel.
      The remainder of the residue was dissolved in hot (60° C)
      water and after cooling the solvent it was also added to
      the separatory funnel. The biphasic system was extracted,

20   and the organic phase was separated and extracted three
      times with water (3 x 100 mℓ). The combined aqueous
      extracts were evaporated in vacuo (60 torr then 1 torr Hg)
      at 40° C to give an oil which was evaporated with anhydrous
      pyridine (50 mℓ). This oil was further dried in vacuo (1

25   torr Hg) at 20° C in the presence of phosphorous pentoxide
      overnight to give N^6-benzoyl-9-(2'-deoxy-2'-fluoro-β-D-
      ribofuranosyl)adenine as a yellow foam (1.08 g, 100 %)
      which contained minor impurities.

      G. N^6-benzoyl-9-[2'-fluoro-5'-O-(4,4'-dimethoxy-
30   trityl)-β-D-ribofuranosyl]adenine.

      N^6-benzoyl-9-(2'-fluoro-b-D-ribofuranosyl)adenine
      (1.08 g, 2.89 mmol) which contained minor impurities was
      dissolved in anhydrous pyridine (20 mℓ) under an argon
      atmosphere, and dry triethylamine (0.52 mℓ, 3.76 mmol) was

35   added followed by addition of 4,4'-dimethoxytrityl chloride
      (1.13 g, 3.32 mmol). After 4 hours of stirring at 20° C the

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reaction mixture was transferred to a separatory funnel and
diethyl ether (40 mℓ) was added to give a white suspension.
This mixture was washed with water three times (3 x 10 mℓ),
the organic phase was separated and dried over magnesium
5   sulfate. Triethylamine (1 mℓ) was added to the solution
and the solvent was evaporated in vacuo (60 torr Hg) at 20°
C to give an oil which was evaporated with toluene (20 mℓ)
containing triethylamine (1 mℓ). This crude product was
purified by column chromatography using silica and ethyl-
10 acetate-triethylamine (99:1, v/v) followed by ethyl
acetate-methanol-triethylamine (80:19:1) to give the
product in two fractions. The fractions were evaporated in
vacuo (60 torr then 1 torr Hg) at 20° C to give a foam which
was further dried in vacuo (1 torr Hg) at 20° C in the
15 presence of sodium hydroxide to give N^6-benzoyl-9-[2'-
fluoro-5'-O-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-
adenine as a foam (1.02 g, 52 %).

        H. N^6-Benzoyl-[2'-fluoro-5'-O-(4,4'-dimethoxy-
              trityl)]adenosine-3'-O-N,N-diisopropyl-β-
20   cyanoethyl phosphoramidite.
              N^6-Benzoyl-9-[2'-fluoro-5'-O-(4,4'-
dimethoxytrityl)-β-D-ribofuranosyl]adenine (1.26 g, 1.89
mmol) was dissolved in anhydrous dichloromethane (13 mℓ)
under an argon atmosphere, diisopropylethylamine (0.82 mℓ,
25 4.66 mmol) was added, and the reaction mixture was cooled
to ice temperature. Chloro(diisopropylamino)-β-
cyanoethoxyphosphine (0.88 mℓ, 4.03 mmol) was added to the
reaction mixture which was allowed to warm to 20° C and
stirred for 3 hours. Ethyl acetate (80 mℓ) and
30 triethylamine (1 mℓ) were added and this solution was
washed with brine solution three times (3 x 25 mℓ). The
organic phase was separated and dried over magnesium
sulfate. After filtration of the solids the solvent was
evaporated in vacuo at 20° C to give an oil which was
35 purified by column chromatography using silica and
hexane-ethyl acetate-triethyl-amine (50:49:1) as eluent.

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Evaporation of the fractions in vacuo at 20° C gave a foam
which was evaporated with anhydrous pyridine (20 ml) in
vacuo (1 torr) at 26° C and further dried in vacuo (1 torr
Hg) at 20° C in the presence of sodium hydroxide for 24 h to
5   give N^6-benzoyl-[2'-deoxy-2'-fluoro-5'-O-(4,4'-
dimethoxytrityl)]adenosine-3'-O-(N,N-diisopropyl-
β-cyanoethylphosphoramidite as a foam (1.05 g, 63 %).

     I.   2'-Deoxy-2'-fluoro-5'-O-(4,4'-
           dimethoxytrityl)-uridine-3'-O-
10   (N,N-diisopropyl-β-cyanoethylphosphoramidite).
           2,2'-Cyclouridine is treated with a solution of
70% hydrogen fluoride/pyridine in dioxane at 120° C for ten
hours to provide after solvent removal a 75% yield of
2'-deoxy-2'- fluorouridine. The 5'-DMT and
15 3'-cyanoethoxydiisopropylphosphoramidite derivitized
nucleoside is obtained by standard literature procedures,
M. J. Gait, ed., *Oligonucleotide Synthesis. A Practical
Approach*, (IRL Press, Washington, DC, 1984) or through the
procedure of Example 1A.

20   J.   2'-Deoxy-2'-fluoro-5'-O-(4,4'-
                 dimethoxytrityl)-cytidine-3'-O-
                 (N,N-diisopropyl-β-cyanoethylphosphoramidite).
                 2'-Deoxy-2'-fluorouridine is converted to the
corresponding cytidine analog via a triazolo intermediate
25 that in turn was aminated The heterocycle is then
protected by selective N^4-benzoylation. The
5'-O-(4,4'-dimethoxy- trityl)-3'-O-(N,N-diisopropyl-β-
cyanoethylphosphoramidite) can be prepared in accordance
with Example 1A.

30   K.   9-(3',5'-[1,1,3,3,-Tetraisopropyldisilox-
                 1,3-diyl]-β-D-arabinofuranosyl)guanine.
                 The 3' and 5' positions of guanosine were
protected by the addition of a TPDS (1,1,3,3-
tetraisopropyldisilox-1,3-diyl) protecting group as per the

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procedure of Robins, M.J., Wilson, J.S., Sawyer, L. and
James, M.N.G. (1983) *Can. J. Chem.*, 61:1911. To a stirred
solution of DMSO (160mls) and acetic anhydride (20.0ml, 212
mmol) was added the TPDS guanosine (21.0 g, 0.040 mol).
5 The reaction was stirred for 36 hrs at room temperature and
then cooled to 0°C. Cold EtOH (400ml, 95%) was added and
the reaction mixture further cooled to -78°C in a dry
ice/acetone bath. NaBH₄ (2.0g, 1.32 mol.eq) was added. The
reaction was allowed to come to -2°C, stirred at -2°C for
10 30 mins, and again cooled to -78°C. This was repeated
twice more. After addition of the NaBH₄ was complete, the
reaction was stirred at ice temperature for 30 mins and
then at RT for 1 hr. The reaction was taken up in EtOAc
(1l) and washed 2X with a saturated NaCl solution. The
15 organic layer was dried over MgSO₄ and evaporated at RT.
The residue was co-evaporated 2X with toluene and purified
by silica gel column chromatography using CH₂Cl₂-MeOH
(90:10) as the eluent. 6.02g of pure product precipitate
from the appropriate column fractions during evaporation of
20 these fraction and an additional 11.49g of product was
obtained as a residue upon evaporated of the fractions.

L. N²-Isobutyryl-9-(2'-O-isobutyryl-3',5'-
[1,1,3,3-tetraisopropyldisilox-1,3-diyl]-ß-D-
arabinofuranosyl)guanine.
25   9-(3',5'-[1,1,3,3-Tetraisopropyldisilox-1,3-
diyl]-ß-D-arabinofuranosyl)guanine (6.5g, 0.01248 mol) was
dissolved in anhydrous pyridine (156ml) under argon. DMAP
(9.15g) was added. Isobutyric anhydride (6.12ml) was
slowly added and the reaction mixture stirred at RT
30 overnight. The reaction mixture was poured into cold sat.
NaHCO₃ (156ml) and stirred for 10 min. The aqueous solution
was extracted 3X with EtOAc (156ml). The organic phase was
washed 3X with sat. NaHCO₃ and evaporated to dryness at RT.
The residue was co-evaporated with toluene at RT. The
35 residue was purified by silica gel column chromatography

using CH$_2$Cl$_2$-acetone (85:15) to yield 5.67g (68%) of
product.

M.   N^2-Isobutyryl-9-(2'-O-isobutyryl-ß-D-arabino-
       furanosyl)guanine.

N^2-Isobutyryl-9-(2'-isobutyryl-3',5'-[1,1,3,3-
tetraisopropyldisilox-1,3-diyl]-ß-D-arabinofuranosyl)-
guanine (9.83g, 0.01476 mol) was dissolved in anhydrous THF
(87.4ml) at RT under argon. 1 M N(nBu)$_4$F in THF (29.52ml, 2
eq.) was added and the mixture stirred for 1/2 hr. The
reaction mixture was evaporated at RT and the residue
purified by silica gel column chromatography using EtOAc-
MeOH (85:15) to yield 4.98g (80%) of product.

N.   N^2-Isobutyryl-9-(2'-O-isobutyryl-3',5'-di-O-
       [tetrahydropyran-2-yl]-ß-D-arabinofuranosyl)-
       guanine.

N^2-Isobutyryl-9-(2'-O-isobutyryl-ß-D-
arabinofuranosyl)guanine (4.9g) was dissolved in anhydrous
1,4-dioxane (98ml) at RT under argon. p-Toluenesulfonic
acid monohydrate (0.97g, .44 eq.) was added followed by
3,4-dihydro-2H-pyran, i.e. DHP, (9.34ml, 8.8 eq.). The
mixture was stirred for 2 hrs then cooled to ice temp and
sat. NaHCO$_3$ (125ml) was added to quench the reaction. The
reaction mixture was extracted 3X with 125ml portions of
CH$_2$Cl$_2$ and the organic phase dried over MgSO$_4$. The organic
phase was evaporated and the residue dissolved in a
minimum, but sufficient amount to yield a clear liquid not
a syrup, volume of CH$_2$Cl$_2$ and dripped into 100 times the
CH$_2$Cl$_2$ volume of hexane. The precipitated was filtered to
give 5.59g (81.5%) of product.

O.   N^2-Isobutyryl-9-(3',5'-di-O-[tetrahydro-
       pyran-2-yl]-ß-D-arabinofuranosyl)guanine.

N^2-Isobutyryl-9-(2'-O-isobutyryl-3',5'-di-O-
[tetrahydropyran-2-yl]-ß-D-arabinofuranosyl)guanine (5.58g)
was dissolved in pyridine:MeOH:H$_2$O (65:30:15, 52ml) at RT.

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The solution was cooled to ice temp and 52ml of 2N NaOH in
EtOH-MeOH (95:15) was added slowly followed by stirring for
2 hrs at ice temp. Glacial AcOH was added to pH6. Sat.
NaHCO$_3$ was then added to pH 7. The mixture was evaporated
5 at RT and the residue co-evaporated with toluene. The
residue was dissolved in EtOAc (150ml) and wash 3X with
sat. NaHCO$_3$. The organic phase was evaporated and the
residue purified by silica gel column chromatography using
EtOAc-MeOH (95:5) to yield 3.85g (78.3%) of product.

10   P.   N^2-Isobutyryl-9-(3',5'-di-O-[tetrahydro-
                  pyran-2-yl]-2'-O-trifluormethylsulfonyl-ß-D-ara-
                  binofuranosyl)guanine.
            N^2-Isobutyryl-9-(3',5'-di-O-[tetrahydropyran-2--
yl]-ß-D-arabinofuranosyl)guanine (3.84g) was dissolved in
15 anhydrous CH$_2$Cl$_2$ (79ml), anhydrous pyridine (5.0ml) and 4-
dimethylaminopyridine (2.93g) at RT under argon. The
solution was cooled to ice temp. and trifluoro-
methanesulfonic anhydride (1.99ml) was slowly added with
stirring. The mixture was stirred for 1 hr then poured
20 into 100ml of sat. NaHCO$_3$. The aqueous phase was extracted
.   3X with cold CH$_2$Cl$_2$. The organic phase was dried over
MgSO$_4$, evaporated and co-evaporated with anhydrous CH$_3$CN at
RT to yield the crude product.

            Q.   N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-3',5'-
25   di-O-[tetrahydropyran-2-yl]-ß-D-
                  ribofuranosyl)guanine.
            The crude product from Example 1-P, i.e. N^2-iso-
butyryl-9-(3',5'-di-O-[tetrahydropyran-2-yl]-2'-O-tri-
fluormethylsulfonyl-ß-D-arabinofuranosyl)guanine, was
30 dissolved in anhydrous THF (113ml) under argon at ice temp.
1M anhydrous N(nBu)$_4$F (dried by co-evaporation with
pyridine) in THF (36.95ml) was added with stirring. After
1 hr a further aliquot of 1M N(nBu)$_4$F in THF (36.95ml) (10
mol. eq. total) was added. The mixture was stirred for 5
35 hrs at ice temp. and stored in a -30°C freezer overnight.

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The reaction mixture was evaporated at RT and the residue
dissolved in CH$_2$Cl$_2$ (160ml) and extracted 5X with deionized
H$_2$O. The organic phase was dried over MgSO$_4$ and evaporated.
The residue was purified by silica gel column

5   chromatography using EtOAc-MeOH (95:5) to yield 5.25g of
product.

R.   N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-ß-D-
ribofuranosyl)guanine
N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-3',5'-di-O-

10   [tetrahydropyran-2-yl]-ß-D-ribofuranosyl)guanine (3.85g)
was dissolved in MeOH (80ml) at RT.   12.32 cm^3 of pre-washed
Dowex 50W resin was added and the mixture stirred at RT for
1 hr. The resin was filtered and the filtrate evaporated
to dryness. The resin was washed with pyridine-

15   triethylamine-H$_2$O (1:3:3) until clear. This filtrate was
evaporated to an oil. The residues from the two filtrates
were combined in H$_2$O (200ml) and washed 3X with CH$_2$Cl$_2$
(100ml). The aqueous phase was evaporated to dryness and
the residue recrystallized from hot MeOH to yield a 0.299g

20   first crop of product as a white powder. The remaining
MeOH solution was purified by silica gel column
chromatography yielding a further crop of 0.783g by elution
with EtOH-MeOH (80:20).

S.   N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-5'-O-

25   [4,4'-dimethoxytrityl]-ß-D-ribofuranosyl)guanine.
N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-ß-D-ribo-
furanosyl)guanine (1.09g) was dissolved in pyridine (20ml)
and triethylamine (0.56ml) at RT under argon.   4,4'-
Dimethoxytrityl chloride (1.20g, 1.15 molar eq.) was added

30   and the mixture stirred at RT for 5 hrs. The mixture was
transferred to a separatory funnel and extracted with Et$_2$O
(100ml). The organic phase was washed 3X with sat. NaHCO$_3$
(70ml portions) and the aqueous phase back extracted 3X
with Et$_2$O. The combined organic phases were dried over

35   MgSO$_4$ and triethylamine (4ml) added to maintain the solution

basic. The solvent was evaporated and the residue purified by silica gel column chromatography. The column was eluted with EtOAc-Et$_3$N (100:1) and then EtOAc-MeOH-Et$_3$N (95:5:1) to yield 1.03g of product. ^{1}H-NMR (DMSO-d$_6$) δ 6.09 (dd, 1,

5 H1', J_{1-2} = 2.61, $J_{1',P}$ = 16.2 Hz); δ 5.28 (ddd, 1, H2', $J_{2'-F}$ = 52.8 Hz); δ 4.38 (m, 1, H3', $J_{3',F}$ = 19.8 Hz).

> T.   N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-5'-O-
> [4,4'-dimethoxytrityl])guanosine-3'-O-N,N-
> diisopropyl-ß-cyanoethyl phosphoramidite.

10   N^2-Isobutyryl-9-(2'-deoxy-2'-fluoro-5'-O-[4,4'-dimethoxytrityl]-ß-D-ribofuranosyl)guanine (0.587g) was dissolved in anhydrous CH$_2$Cl$_2$ (31ml) and diisopropyl-ethylamine (0.4ml) at RT under argon. The solution was cooled to ice temp and chloro(diisopropylamino)-ß-

15 cyanoethoxyphosphine (0.42ml) was slowly added. The reaction was allowed to warm to RT and stirred for 3.5 hrs. CH$_2$Cl$_2$-Et$_3$N (100:1, 35ml) was added and the mixture washed 1X with sat. NaHCO$_3$ (6ml). The organic phase was dried over MgSO$_4$ and evaporated at RT. The residue was purified by

20 silica gel column chromatography using Hex-EtOAc-Et$_3$N (75:25:1) for 2 column volumes, then Hex-EtOAc-Et$_3$N (25:75:1) and finally EtOAc-Et$_3$N. The product containing fractions were pooled and evaporated at RT. The resulting oil was co-evaporated 2X with CH$_3$CN and placed on a vacuum

25 pump overnight to dry. The resulting white solid was dissolved in CH$_2$Cl$_2$ (3ml) and dripped into stirring hexane (300ml). The resulting precipitate was filtered and dried on a vacuum pump to yield 0.673g (88%) of product. ^{31}P-NMR (CDCl$_3$) δ 150.5, 151.5.

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EXAMPLE 2 - Preparation of 2'-Deoxy-2'-cyano Modified Oligonucleotides.

      A. N^6-Benzoyl-[2'-deoxy-2'-cyano-5'-O-(4,4'-dimethoxytrityl)] adenosine-3'-O-

5   **(N,N-diisopropyl-β-cyanoethylphosphoramidite).**

      2'-Deoxy-2'-cyanoadenosine is prepared by the free radical replacement of the 2'-iodo group of 2'-deoxy-2'-iodo-3',5'-O-(disiloxytetraisopropyl)-N6-benzoyladenosine according to a similar procedure

10 described by K.E.B. Parkes and K. Taylor, *Tetrahedron Letters* 29:2995-2996 (1988). 2'-Deoxy-2'-iodoadenosine was prepared by R. Ranganathan as described in *Tetrahedron Letters* 15:1291-1294 (1977), and disilyated as described by W.T. Markiewicz and M. Wiewiorowski in *Nucleic Acid*

15 *Chemistry*, Part 3, pp. 222-231, Townsend, L.B.; Tipson, R.S. eds. (J. Wiley and Sons, New York, 1986). This material is treated with hexamethylditin, AIBN, and t-butylisocyanate in toluene to provide protected 2'-deoxy-2'-cyanoadenosine. This material, after selective

20 deprotection, is converted to its 5'-DMT-3'-phosphoramidite as described in Example 1A.

      B. 2'-Deoxy-2'-cyano-5'-O-(4,4'-dimethoxytrityl)-uridine-3'-O-(N,N-diisopropyl-β-cyanoethyl phos-phoramidite).

25   2'-Deoxyuridine (or 5-methyluridine), 3',5'-disilylated as described above, is converted to the 2'-iodo derivative by triphenylphosphonium methyl iodide treatment as described by K.E.B. Parkes and K. Taylor, *Tetrahedron Letters* 29:2995-2996 (1988). Application of

30 free radical reaction conditions as described by K.E.B. Parkes and K. Taylor, *Tetrahedron Letters* 29:2995-2996 (1988), provides the 2'-cyano group of the protected nucleoside. Deprotection of this material and subsequent conversion to the protected monomer as described above

35 provides the requisite nucleic acid synthesizer material.

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C. 2'-Deoxy-2'-cyano-5'-O-(4,4'-dimethoxytrityl)-
cytidine-3'-O-(N,N-diisopropyl-β-cyanoethyl
phosphoramidite).

2'-Deoxy-2'-iodocytidine is obtained from the
5 corresponding above described uridine compound via a
conventional keto to amino conversion.

D. 2'-Deoxy-2'-cyano-5'-O-(4,4'-dimethoxytrityl)-
guanosine -3'-O-(N,N-diisopropyl-β-cyanoethyl
phos-phoramidite).

10   2'-Deoxy-2'-cyanoguanosine is obtained by the
displacement of the triflate group in the 2'-up position
(arabino sugar) of 3',5'-disilylated N2-isobutrylguanosine.
Standard deprotection and subsequent reprotection provides
the title monomer.

15 **EXAMPLE 3 - Preparation of 2'-Deoxy-2'-(trifluoromethyl)
Modified Oligonucleotides.**

The requisite 2'-deoxy-2'-trifluromethyribosides
of nucleic acid bases A, G, U(T), and C are prepared by
modifications of a literature procedure described by Q.-Y.
20 Chen and S.W. Wu in the *Journal of Chemical Society Perkin
Transactions* 2385-2387 (1989). Standard procedures, as
described in Example 1A, are employed to prepare the 5'-DMT
and 3'-phosphoramidites as listed below.

A. N^6-Benzoyl-[2'-deoxy-2'-trifluoromethyl-5'-O-
25   (4,4'-dimethoxytrityl)]adenosine-3'-O-(N,N-di-
isopropyl-β-cyanoethyl phosphoramidite).

B. 2'-Deoxy-2'-trifluoromethyl-5'-O-
(4,4'-dimethoxytrityl)uridine-3'-O-(N,N-
diisopropyl-β-cyanoethyl-phosphoramidite).

30   C. 2'-Deoxy-2'-trifluoromethyl-5'-O-
(4,4'-dimethoxytrityl)cytidine-3'-O-(N,N-
diisopropyl-β-cyanoethyl-phosphoramidite).

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            D. 2'-Deoxy-2'-trifluoromethyl-5'-O-
            (4,4'-dimethoxytrityl)guanosine-3'-O-(N,N-
            diisopropyl-β-cyano-ethylphosphoramidite).

        EXAMPLE 4 - Preparation of 2'-Deoxy-2'-(trifluoromethoxy)
5   Modified Oligonucleotides.
            The requisite 2'-deoxy-2'-O-trifluoromethyl-
        ribosides of nucleic acid bases A, G, U(T), and C are
        prepared by modifications of literature procedures
        described by B.S. Sproat, et al., *Nucleic Acids Research*
10 18:41-49 (1990) and H. Inoue, et al., *Nucleic Acids
        Research* 15:6131-6148 (1987). Standard procedures, as
        described in Example 1A, are employed to prepare the 5'-DMT
        and 3'-phosphoramidites as listed below.
            A. N6-Benzoyl-[2'-deoxy-2'-(trifluoromethoxy)-
15   5'-O-(4,4'-dimethoxytrityl)]adenosine-3'-O-(N,N-
            diisopropyl-β-cyanoethylphosphoramidite).

            B. 2'-Deoxy-2'-(trifluoromethoxy)- 5'-O-(4,4'-
            dimethoxytrityl)uridine-3'-O-(N,N-diisopropyl-β-c
            yanoethylphosphoramidite).

20   C. 2'-Deoxy-2'-(trifluoromethoxy)-5'-O-(4,4'-
            dimethoxytrityl)cytidine-3'-O-(N,N-diisopropyl-β-
            cyanoethylphosphoramidite).

            D. 2'-Deoxy-2'-(trifluoromethoxy)-5'-O-(4,4'-
            dimethoxytrityl)guanosine-3'-O-(N,N-diisopropyl-β
25   -cyanoethylphosphoramidite).

        EXAMPLE 5 - Preparation of 2'-Deoxy-2'-(1-proproxy)
        Modified Oligonucleotides.
            The requisite 2'-deoxy-2'-O-propyl ribosides of
        nucleic acid bases A, G, U(T), and C are prepared by
30 modifications of literature procedures described by B.S.
        Sproat, et al., *Nucleic Acids Research* 18:41-49 (1990) and

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H. Inoue, et al., *Nucleic Acids Research* 15:6131-6148
(1987). Standard procedures, as described in Example 1A,
are employed to prepare the 5'-DMT and 3'-phosphoramidites
as listed below.

5   A. N^6-Benzoyl-[2'-deoxy-2'-(1-proproxy)-5'-O-
         (4,4'-dimethoxytrityl)]adenosine-3'-O-(N,N-
         diisopropyl-β-cyanoethyl phosphoramidite).

         B. 2'-Deoxy-2'-(1-proproxy)-5'-O-(4,4'-dimethoxy-
         trityl)uridine-3'-O-(N,N-diisopropyl-β-cyano-
10   ethylphosphoramidite).

         C. 2'-Deoxy-2'-(1-proproxy)-5'-O-(4,4'-dimethoxy-
         trityl)cytidine-3'-O-(N,N-diisopropyl-β-cyano-
         ethylphosphoramidite).

         D. 2'-Deoxy-2'-(1-proproxy)-5'-O-(4,4'-dimethoxy-
15   trityl)guanosine-3'-O-(N,N-diisopropyl-β-cyano-
         ethylphosphoramidite).

**EXAMPLE 6 - Preparation of 2'-Deoxy-2'-(vinyloxy) Modified
Oligonucleotides.**
         The requisite 2'-deoxy-2'-O-vinyl ribosides of
20 nucleic acid bases A, G, U(T), and C are prepared by
modifications of literature procedures described by B.S.
Sproat, et al., *Nucleic Acids Research* 18:41-49 (1990) and
H. Inoue, et al., *Nucleic Acids Research* 15:6131-6148
(1987). In this case 1,2-dibromoethane is coupled to the
25 2'-hydroxyl and subsequent dehydrobromination affords the
desired blocked 2'-vinyl nucleoside. Standard procedures,
as described in Example 1A, are employed to prepare the
5'-DMT and 3'-phosphoramidites as listed below.

         A. N^6-Benzoyl-[2'-deoxy-2'-(vinyloxy)-5'-O-(4,4'-
30   dimethoxytrityl)]adenosine-3'-O-(N,N-diisopropyl-
         β-cyanoethylphosphoramidite).

B. 2'-Deoxy-2'-(vinyloxy)-5'-O-(4,4'-dimethoxy-
trityl)uridine-3'-O-(N,N-diisopropyl-β-cyano-
ethylphosphoramidite).

C. 2'-Deoxy-2'-(vinyloxy)-5'-O-(4,4'-dimethoxy-
5   trityl)cytidine-3'-O-(N,N-diisopropyl-β-cyano-
ethylphosphoramidite).

D. 2'-Deoxy-2'-(vinyloxy)-5'-O-(4,4'-dimethoxy-
trityl)guanosine-3'-O-(N,N-diisopropyl-β-cyano-
ethylphosphoramidite).

10 EXAMPLE 7 - Preparation of 2'-Deoxy-2'-(allyloxy) Modified
Oligonucleotides.

The requisite 2'-deoxy-2'-O-allyl ribosides of
nucleic acid bases A, G, U(T), and C are prepared by
modifications of literature procedures described by B.S.
15 Sproat, et al., *Nucleic Acids Research* 18:41-49 (1990) and
H. Inoue, et al., *Nucleic Acids Research* 15:6131-6148
(1987). Standard procedures, as described in Example 1A,
are employed to prepare the 5'-DMT and 3'-phosphoramidites
as listed below.

20   A. N[6]-Benzoyl-[2'-deoxy-2'-(allyloxy)-5'-O-(4,4'-
dimethoxytrityl)]adenosine-3'-O-(N,N-
diisopropyl-β-cyanoethylphosphoramidite).

B. 2'-Deoxy-2'-(allyloxy)-5'-O-(4,4'-dimethoxy-
trityl)-uridine-3'-O-(N,N-diisopropyl-β-cyano-
25   ethylphosphoramidite).

C. 2'-Deoxy-2'-(allyloxy)-5'-O-(4,4'-dimethoxy-
trityl)-cytidine-3'-O-(N,N-diisopropyl-β-cyano-
ethylphosphoramidite).

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D. 2'-Deoxy-2'-(allyloxy)-5'-O-(4,4'-dimethoxy-
   trityl)-guanosine-3'-O-(N,N-diisopropyl-β-cyano-
   ethylphosphoramidite).

EXAMPLE 8 - Preparation of 2'-Deoxy-2'-(methylthio),
5 **(methylsulfinyl) and (methylsulfonyl) Modified**
   Oligonucleotides
           A.   2'-Deoxy-2'-Methylthiouridine
           2,2'-Anhydrouridine (15.5g, 68.2 mmol) [Rao, T.S.
   and Reese, C.B. (1989) *J. Chem. Soc., Chem. Commun.*, 997],
10 methanethiol (15.7g, 327mmol), 1,1,3,3-tetramethylguanidine
   (39.2g, 341mmol) and dimethylforamide (150ml) were heated
   together at 60°C. After 12 hr, the reaction mixture was
   cooled and concentrated under reduced pressure. The
   residual oil was purified by flash column chromatography on
15 silica gel (300g). Concentration of the appropriate
   fractions, which were eluted with CH_2Cl_2-MeOH (9:1, v/v),
   and drying of the residue under high vacuum gave 2'-deoxy-
   2'-methylthiouridine as a pale yellow solid (14.11g,
   75.4%). Attempts to crystallize the solids from ethanol-
20 hexanes (as reported by Imazawa, M., Ueda, T., and Ukita,
   T. (1975) *Chem. Pharm. Bull.*, 23:604) failed and the
   material turned into a hygroscopic foam.
   ^{1}H NMR (Me2SO-d6) δ 2.0 (3H, s, SC$\underline{H}_3$), 3.34 (1H, dd, $J_{3',2'}$
   =54.Hz, 2'$\underline{H}$), 3.59 (2H, br m, 5'C$\underline{H}_2$), 3.84 (1H, m, 4'$\underline{H}$), 4.2
25 (1H, dd, $J_{3',4'}$ = 2.2 Hz, 3'$\underline{H}$), 5.15 (1H, t, 5'O$\underline{H}$), 5.62 (1H,
   t, 3'O$\underline{H}$), 5.64 (1H, d, $J_{C6,C5}$ = 8.2Hz), 6.02 (1H, d, $J_{1',2'}$ =
   6Hz, 1'$\underline{H}$), 7.82 (1H, d, $J_{C5,C6}$ = 8.2 Hz, C$_6\underline{H}$), 11.38 (1H, br
   s, N$\underline{H}$).

           B.   2,2'-Anhydro-5-Methyluridine
30   A mixture of 5-methyluridine (16.77g, 69.2mmol),
   diphenyl carbonate (17.8g, 83.1mmol) and sodium bicarbonate
   (100mg) in hexamethylphosphoramide (175ml) was heated to
   150°C with stirring until evolution of CO_2 ceased
   (approximately 1hr). The reaction mixture was cooled and
35 then poured into diethylether (1l) while stirring to

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furnish a brown gum. Repeated washings with diethylether
(4 X 250ml) furnished a straw colored hygroscopic powder.
The solid was purified by short column chromatography on
silica gel (400g). Pooling and concentration of
5   appropriate fractions, which were eluted with CH$_2$Cl$_2$-MeOH
(85:15, v/v) furnished the title compound as a straw
colored solid (12g, 77.3%) which crystallized from EtOH as
long needles, m.p. 226-227°C.

C. 2'-Deoxy-2'-Methylthio-5-Methyluridine

10   2.2'-Anhydro-5-methyluridine (17.02g, 70.6mmol),
methanethiol (16.3g, 339mmol), 1,1,3,3-tetramethylguanidine
(40.6g, 353mmol), and dimethylformamide (150ml) were heated
together at 60°C. After 12 hr, the products were cooled
and concentrated under reduced pressure. The residual oil
15   was purified by short silica gel (300g) column
chromatography. Concentration of appropriate fractions,
which were eluted with CH$_2$Cl$_2$-MeOH (93:7, v/v), furnished
the title compound as a white foam (15.08g, 74.1%).
Crystallization from EtOH-CH$_2$Cl$_2$ furnished white needles.

D. 2'-Deoxy-2'-Methylsulfinyluridine

20   To a stirred solution of 2'-deoxy-
2'methylthiouridine (1g, 3.65mmol) in EtOH (50ml) was added
a solution of m-chloroperbenzoic acid (50%, 1.26g, 3.65mmol
in 50ml EtOH) over a period of 45 min at 0°C. The solvent
25   was removed under vacuum and the residue purified by short
silica gel (30g) column chromatography. Concentration of
appropriate fractions, which were eluted with CH$_2$Cl$_2$-MeOH
(75:25, v/v), afforded the title compound as a white solid
(0.65g, 61.4%). Crystallization from EtOH furnished white
30   granules, m.p. 219-221°C.
^{1}H NMR (Me$_2$SO-d$_6$) δ 2.5(3H, s, SOCH$_3$), 3.56 (2H, br s,
5'CH$_2$), 3.8 (1H, m, 4'H), 3.91 (1H, m, 2'H), 4.57 (1H, m,
3'H), 5.2 (1H, br s, 5'OH), 5.75 (1H, d, C$_5$H), 6.19 (1H, d,
3'OH), 6.35 (1H, d, 1'H), 7.88 (1H, d, C$_6$H), 11.43 (1H, br
35   s, NH).

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E.   2'-Deoxy-2'-Methylsulfonyluridine

To a stirred solution of 2'-deoxy-2'-
methyluridine (1g, 3.65mmol) in EtOH (50 ml) was added m-
chloroperbenzoic acid (50%, 3.27g, 14.6mmol) in one portion
5   at room temperature. After 2hr., the solution was filtered
to collect a white precipitate, which on washing (2 X 20ml,
EtOH and 2 X 20ml Et$_2$O) and drying furnished the title
compound as a fine powder (0.76g, 68%), m.p. 227-228°C.
^{1}H NMR (Me$_2$SO-d$_6$) δ 3.1 (3H, s, SO$_2$CH$_3$), 3.58 (2H, m, 5'CH$_2$),
10   3.95 (1H, m, 2'H), 3.98 (1H, m, 4'H), 4.5 (1H, br s, 3'H),
5.2 (1H, br s, 5'OH), 5.75 (1H, d, C$_5$H), 6.25 (1H, d, 3'OH),
6.5 (1H, d, 1'H), 7.8 (1H, d, C$_6$H), 11.45 (1H, br s, NH).

F.   2'-Deoxy-5-O-(4,4'-Dimethoxytrityl)-2'-
Methylthiouridine

15   To a stirred solution of 2'-deoxy-2'-
methylthiouridine (1.09g, 4mmol)) in dry pyridine (10ml)
was added 4,4'-dimethoxytritylchloride (1.69g, 5mmol) and
4-dimethylaminopyridine (50mg) at room temperature. The
solution was stirred for 12hr and the reaction quenched by
20   adding MeOH (1ml). The reaction mixture was concentrated
under vacuum and the residue dissolved in CH$_2$Cl$_2$ (100ml),
washed with sat. aq. NaHCO$_3$ (2 X 50ml), sat. aq. NaCl (2 X
50ml), and dried (MgSO$_4$). The solution was concentrated
under vacuum and the residue purified by silica gel (30g)
25   column chromatography. Elution with CH$_2$Cl$_2$-
MeOH:triethylamine (89:1:1, v/v) furnished the title
compound as homogeneous material. Pooling and
concentration of appropriate fractions furnished the 5'-O-
DMT nucleoside as a foam (1.5g, 66.5%).
30   ^{1}H NMR (MeSO-d$_6$) δ 2.02 (3H, s, SCH$_3$), 3.15-3.55 (1H, m,
2'CH), 3.75 (6H, s, 2 OCH$_3$), 3.97 (1H, m, 4'H), 4.24 (1H, m,
3'H), 5.48 (1H, d, C$_5$H), 5.73 (1H, d, 3'-OH), 6.03 (1H, d,
C1'H), 6.82-7.4 (13H, m, ArH), 6.65 (1H, d, C$_6$H), 11.4 (1H,
br s, NH).

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G.   2'-Deoxy-3'-O-[(N,N-diisopropyl)-O-β-
cyanoethylphosphoramide]-5'-O-(4,4'-
dimethoxytrityl)-2'-Methylthiouridine

To a stirred solution of 2'-deoxy-5'-O-(4,4'-

5   dimethoxytrityl)-2'-methylthiouridine (1.5g, 2.67mmol) in
dry THF (25ml) was added diisopropylethylamine (1.4ml,
8mmol) and the solution cooled to 0°C. N,N-diisopropyl-β-
cyanoethylphosphoramidic chloride (1.26ml, 5.34mmol) was
added dropwise over a period of 15 min. The reaction

10   mixture was then stirred at room temperature for 2 hr.
EtOAc (100ml, containing 1% triethylamine) was added and
the solution washed with sat NaCl (2 X 50ml) and the
organic layer dried over MgSO₄. The solvent was removed
under pressure and the residue purified by short silica gel

15   (30g) column chromatography. Elution with
CH₂Cl₂:MeOH:triethylamine (98:1:1, v/v) furnished the
product as a mixture of diastereoisomers. Evaporation of
the appropriate fractions provided the title compound as a
foam (1.32g, 64.7%).

20   ¹H NMR (CDCl₃) δ 2.0 and 2.02 (3H, 2s, SC$\underline{H}_3$), 5.3 and 5.35
(1H, 2d, C₅$\underline{H}$), 6.23 (1H, d, 1'$\underline{H}$), 7.8 and 7.78 (1H, 2d, C₆$\underline{H}$)
and other protons. ³¹P NMR (CDCl₃) δ 151.68 and 152.2 ppm.

H.   2'-Deoxy-3',5'-di-O-Acetyl-2'-
Methylthiouridine

25   2'-Deoxy-2'-methylthiouridine (5.0g, 18.24 mmol)
and acetic anhydride (5.6ml, 54.74mmol) were stirred
together in dry pyridine (30ml) at room temperature for 12
hr. The products were then concentrated under reduced
pressure and the residue obtained was purified by short

30   silica gel column chromatography. The appropriate
fractions, which were eluted with CH₂Cl₂:MeOH (9:1, v/v),
were combined, evaporated under reduced pressure and the
residue was crystallized from EtOH to give the title
compound (6.0g, 91.8%) as white needles, m.p. 132°C.

35   ¹H NMR (CDCl₃) δ 2.17 (3H, s, SC$\underline{H}_3$), 2.20 (6H, s, 2 COC$\underline{H}_3$),
3.40 (1H, t, 2'$\underline{H}$), 4.31-4.40 (3H, m, 4',5'$\underline{H}$), 5.31 (1H, m,

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3'H), 5.80 (1H, d, C₅H), 6.11 (1H, d, 1'H), 7.45 (1H, d, C₆H), 8.7 (1H, br s, NH).

I.   2'-Deoxy-3',5'-di-O-Acetyl-4-(1,2,4-triazol-1-yl)-2'-Methylthiouridine

5   Triethylamine (8.4ml, 60.3mmol) and phosphoryl chloride (1.2ml, 12.9mmol) were added to a stirred solution of 2'-deoxy-3',5'-di-O-acetyl-2'-methylthiouridine (4.6g, 13mmol) in CH₃CN (50ml). 1,2,4-Triazole (4.14g, 59.9mmol) was then added and the reactants were stirred together at
10   room temperature. After 16hr, triethylamine-water (6:1, v/v; 20ml) followed by sat. aq. NaHCO₃ (100ml) were added to the products and the resulting mixture was extracted with CH₂Cl₂ (2 X 100ml). The organic layer was dried (MgSO₄) and evaporated under reduced pressure. The residue was
15   purified by short silica gel column chromatography. The appropriate fractions, which were eluted with CH₂Cl₂:MeOH (9:1, v/v), were evaporated under vacuum and the residue was crystallized from EtOH to give the title compound (3.01g, 56.4%) as pale needles, m.p. 127-130°C.
20   ¹H NMR (CDCl₃) δ 2.18 (6H, s, 2 COCH₃), 2.30 (3H, s, SCH₃), 3.67 (1H, m, 2'H), 4.38-4.50 (3H, m, 4',5'H), 5.17 (1H, t, 3'H), 6.21 (1H, d, 1'H), 7.08 (1H, d, C₅H), 8.16 (1H,s, CH), 8.33 (1H, d, C₆H), 9.25 (1H, s, CH).

J.   2'-Deoxy-2'-Methylthiocytidine

25   2'-Deoxy-3',5'-di-O-acetyl-4-(1,2,4-triazol-1-yl)-2'-methylthiouridine (3.0g, 7.5mmol) was dissolved in a saturated solution of ammonia in MeOH (70ml) and the solution was stirred at room temperature in a pressure bottle for 3 days. The products were then concentrated
30   under reduced pressure and the residue was crystallized from EtOH:CH₂Cl₂ to give the title compound (1.06g, 51.7%) as crystals, m.p. 201°C.
       ¹H NMR (Me₂SO-d₆) δ 1.95 (3H, s, SCH₃), 3.36 (1H, m, 2'H), 3.55 (2H, m, 5'CH₂), 3.82 (1H, m, 4'H), 4.18 (1H, dd, 3'H),
35   5.75 (1H, d, C₅H), 6.1 (1H, d, 1'H), 7.77 (1H, d, C₆H).

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Anal. calcd. for $C_{10}H_{15}N_3O_4S$: C, 43.94; H, 5.53; N, 15.37; S, 11.73. Found, C, 44.07; H, 5.45; N, 15.47; S, 11.80.

K.   2'-Deoxy-N⁴-Benzoyl-2'-Methylthiocytidine

To a stirred solution of 2'-deoxy-2'-
5 methylthiocytidine (0.86g, 3.15mmol) in dry pyridine (20
ml) was added trimethylchlorosilane (2.0ml, 15.75mmol), and
stirring continued for 15 min. Benzoyl chloride (2.18ml,
18.9mmol) was added to the solution followed by stirring
for 2 hr. The mixture was then cooled in an ice-bath and
10 MeOH (10ml) was added. After 5 mins., NH₄OH (20ml, 30% aq.)
was added and the mixture stirred for 30 min. The reaction
mixture was then concentrated under vacuum and the residue
purified by short silica gel (70g) column chromatography.
Elution with CH_2Cl_2:MeOH (9:1, v/v), pooling of appropriate
15 fractions and evaporation furnished the title compound
(0.55g, 46.6%) which crystallized from EtOH as needles,
m.p. 193-194°C.

L.   N⁴-Benzoylamino-1-[2-Deoxy-5-(4,4'-
Dimethoxytrityl)-2-Methylthio-β-D-
20   Ribofuranosyl]pyrimidin-3(2H)-one (or 2'-Deoxy-
N⁴-Benzoyl-5'-(4,4'-Dimethoxytrityl)-2'-
Methylthiocytidine)

To a stirred solution of 2'-deoxy-N⁴-benzoyl-2'-
methylthiocytidine (0.80g, 2.12mmol) in dry pyridine (10ml)
25 was added 4,4'-dimethoxytrityl chloride (1.16g, 3.41mmol)
and 4-dimethylaminopyridine (10mg) at room temperature.
The solution was stirred for 2 hr and the products
concentrated under vacuum. The residue was dissolved in
CH_2Cl_2 (70ml), washed with sat. NaHCO₃ (50ml), sat. NaCl (2
30 X 50ml), dried (MgSO₄) and evaporated under reduced
pressure. The residue was purified by short silica gel
(50g) column chromatography. Elution with
CH_2Cl_2:triethylamine (99:1, v/v), pooling and concentration
of appropriate fractions furnished the title compound
35 (1.29g, 90%) as a white foam.

^{1}H NMR (DMSO-d$_6$) δ 2.1 (3H, s, SC$\underline{H}_3$), 3.5 (1H, m, 2'$\underline{H}$), 3.75
(6H, s, OC$\underline{H}_3$), 4.15 (1H, m, 4'$\underline{H}$), 4.4 (1H, t, 3'$\underline{H}$), 5.74
(1H, br d, 3'O$\underline{H}$), 6.15 (1H, d, Cl'$\underline{H}$), 6.8-8.0 (25H, m, Ar$\underline{H}$,
and C$_5\underline{H}$), 8.24 (1H, d, C$_6\underline{H}$), 11.3 (1H, br s, N$\underline{H}$).

5   **M.   2'-Deoxy-N^4-Benzoyl-3-O-[(N,N-Diisopropyl)-
        β-Cyanoethylphosphoramide]-5'-O'(4,4'-
        Dimethoxytrityl)-2'-Methylthiocytidine**
             2'-Deoxy-N^4-benzoyl-5'-(4,4'-dimethoxytrityl)-2'-
methylthiocytidine (1.41g, 2.07mmol) was treated with
10   diisopropylethylamine (1.4ml, 8mmol) and N,N-diisopropyl-
β-cyanoethylphophoramide chloride (1.26ml, 5.34mmol) in dry
THF (25ml) as described in Example 8-G above. The crude
product was purified by short silica gel (50g)
chromatography to furnish the title compound on elution
15   with CH$_2$Cl$_2$:hexanes:triethylamine (89:10:1, v/v). The
appropriate fractions were mixed and evaporated under
pressure to give the title compound (1.30g, 71%) as a white
foam (mixture of diastereoisomers).
^{1}H NMR (CDCl$_3$) δ 2.31 (3H, s, SC$\underline{H}_3$), 3.45-3.7 (3H, m, 2'$\underline{H}$
20   and 5'C$\underline{H}_2$), 3.83 (6H, s, OC$\underline{H}_3$), 4.27-4.35 (1H, m, 4'$\underline{H}$), 4.6-
4.8 (1H, m, 3'$\underline{H}$), 6.35 (1H, 2d, 1'$\underline{H}$), 6.82-7.8 (25H, m, Ar$\underline{H}$
and C$_5\underline{H}$), 8.38 and 8.45 (1H, 2d, C$_6\underline{H}$) and other protons. ^{31}P
NMR δ 151.03 and 151.08 ppm.

        N.   2'-Deoxy-2'-Methylsulfinylcytidine
25   2'-Deoxy-2'-methylthiocytidine of Example 8-J was
treated as per the procedure of Example 8-D to yield the
title compound as a mixture of diastereoisomers having a
complex ^{1}H NMR spectrum.

        O.   2'-Deoxy-2'-Methylsulfonylcytidine
30   2'-Deoxy-2'-methylthiocytidine of Example 8-J was
treated as per the procedure of Example 8-E to yield the
title compound.

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P.   N⁶-Benzoyl-3',5'-di-O-[Tetrahydropyran-2-
yl]-2'-Deoxy-2'-Methylthioadenosine

    N^6-Benzoyl-9-[2'-O-trifluoromethylsulfonyl-3',5'-
di-O-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]adenine

5   from Example 1-D is prepared by treatment with methanethiol
in the presence of tetramethylguanidine to yield the title
compound.

Q.   N^6-Benzoyl-2'-Deoxy-2'-Methylthioadenosine

    N^6-Benzoyl-3',5'-di-O-(tetrahydropyran-2-yl)-2'-

10   deoxy-2'-methylthioadenosine from Example 8-P is treated as
per Example 1-F to yield the title compound.

R.   N^6-Benzoyl-2'-Deoxy-2'-Methylsulfinyl-
adenosine

    N^6-Benzoyl-2'-deoxy-2'-methylthioadenosine from

15   Example 8-Q was treated as per the procedure of Example 8-
D to yield the title compound.

S.   N^6-Benzoyl-2'-Deoxy-2'-Methylsulfonyl-
adenosine

    N^6-Benzoyl-2'-deoxy-2'-methylthioadenosine from

20   Example 8-Q was treated as per the procedure of Example 8-
E to yield the title compound.

T.   N^2-Isobutyryl-3',5'-di-O-(tetrahydropyran-2-
yl)-2'-Deoxy-2'-Methylthioguanosine

    N^2-Isobutyryl-9-(3',5'-di-O-[tetrahydropyran-2-

25   yl]-2'-O-trifluoromethylsulfonyl-β-D-arabinofuranosyl)-
guanine from Example 1-P is treated with methanethiol in
the presence of 1,1,3,3-tetramethylguanidine to yield the
title compound.

U.   N^2-Isobutyryl-2'-Deoxy-2'-Methylthioguanosine

30   N^2-Isobutyryl-3',5'-di-O-(tetrahydropyran-2-yl)-
2'-deoxy-2'-methylthioguanosine is treated as per Example
1-R to yield the title compound.

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**V.   N²-Isobutyryl-2'-Deoxy-2'-Methylsulfinyl-
guanosine**

N²-Isobutyryl-2'-Deoxy-2'-methylthioguanosine from
Example 8-U was treated as per the procedure of Example 8-
5   D to yield the title compound.

**W.   N²-Isobutyryl-2'-Deoxy-2'-Methylsulfonyl-
guanosine**

N²-Isobutyryl-2'-Deoxy-2'-methylthioguanosine from
Example 8-U was treated as per the procedure of Example 8-
10   E to yield the title compound.

**X.   2'-Deoxy-5-O-(4,4'-Dimethoxytrityl)-2'-
Methylsulfinyluridine**

2'-Deoxy-2'-methylsulfinyluridine from Example 8-
D above is treated as per Example 8-F to yield the title
15   compound.

**Y.   2'-Deoxy-3'-O-[(N,N-Diisopropyl)-O-ß-
cyanoethylphosphoramide]-5'-O-(4,4'-
Dimethoxytrityl)-2'-Methylsulfinyluridine**

2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-
20   methylsulfinyluridine is treated as per Example 8-G to
yield the title compound.

**Z.   N⁶-Benzoyl-2'-Deoxy-5-O-(4,4'-
Dimethoxytrityl)-2'-Methylthioadenosine**

N⁶-benzoyl-2'-Deoxy-2'-methylthioadenosine from
25   Example 8-Q above is treated as per Example 8-F to yield
the title compound.

**AA.   N⁶-Benzoyl-2'-Deoxy-3'-O-[(N,N-Diisopropyl)-
O-ß-Cyanoethylphosphoramide]-5'-O-(4,4'-
Dimethoxytrityl)-2'-Methylthioadenosine**

30   N⁶-benzoyl-2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-
2'-methylthioadenosine is treated as per Example 8-G to
yield the title compound.

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BB.　2'-Deoxy-N^2-Isobutyryl-5-O-(4,4'-
Dimethoxytrityl)-2'-Methylthioguanosine
2'-Deoxy-N^2-isobutyryl-2'-methylthioguanosine from
Example 8-U above is treated as per Example 8-F to yield
5　the title compound.

CC.　2'-Deoxy-N^2-Isobutyryl-3'-O-[(N,N-
Diisopropyl)-O-ß-Cyanoethylphosphoramide]-5'-O-
(4,4'-Dimethoxytrityl)-2'-Methylthioguanosine
2'-Deoxy-N^2-isobutyryl-5'-O-(4,4'-
10　dimethoxytrityl)-2'-methylthioguanosine is treated as per
Example 8-G to yield the title compound.

DD.　2'-Deoxy-5-O-(4,4'-Dimethoxytrityl)-2'-
Methylsulfonyluridine
2'-Deoxy-2'-methylsulfonyluridine from Example 8-
15　E above is treated as per Example 8-F to yield the title
compound.

EE.　2'-Deoxy-3'-O-[(N,N-Diisopropyl)-O-ß-
Cyanoethylphosphoramide]-5'-O-(4,4'-
Dimethoxytrityl)-2'-Methylsulfinyluridine
20　　　　2'-Deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-
methylsulfinyluridine is treated as per Example 8-G to
yield the title compound.

EXAMPLE 9 - Chemical conversion of an thymine or cytosine
(pyrimidine type base) to its ß-D-2'-deoxy-2'-substituted
25　erythro-pentofuranosyl nucleoside; 2'-substituted ribosyl-
ation).
The thymine or cytosine type analogs are tri-
methylsilylated under standard conditions such as hexa-
methyldisilazane (HMDS) and an acid catalyst (ie. ammonium
30　chloride) and then treated with 3,5-O-ditoluoyl-2-deoxy-2-
substituted-α-D-erythro-pentofuranosyl chloride in the
presence of Lewis acid catalysts (ie. stannic chloride,
iodine, boron tetrafluoroborate, etc.). A specific

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procedure has recently been described by J. N. Freskos,
Nucleosides & Nucleotides
8:1075-1076 (1989) in which copper (I) iodide is the
catalyst employed.

5   **EXAMPLE 10 - Chemical conversion of an adenine or guanine
(purine type base) to its β-D-2'-deoxy-2'-substituted
erythro-pentofuranosyl nucleoside; 2'-substituted ribosyl-
ation).**

       The protected purine type analogs are converted
10 to their sodium salts via sodium hydride in acetonitrile
and are then treated with 3,5-O-ditoluoyl-2-deoxy-2-
substituted-α-D-erythro-pentofuranosyl chloride at ambient
temperature. A specific procedure has recently been
described by R.K. Robins et al., *Journal of American
15 Chemical Society* 106:6379 (1984).

    **EXAMPLE 11 - Conversion of 2'-deoxy-2-substituted
thymidines to the corresponding 2'-deoxy-2'-substituted
cytidines (chemical conversion of an pyrimidine type 4-keto
group to an 4-amino group).**
20   The 3',5'-sugar hydroxyls of the 2'modified
nucleoside types are protected by acyl groups such as
toluoyl, benzoyl, p-nitrobenzoyl, acetyl, isobutryl,
trifluoroacetyl, etc. using standards conditions of the
acid chlorides or anhydrides and pyridine/dimethylamino-
25 pyridine solvent and catalyst. The protected nucleoside is
now chlorinated with thionyl chloride or phosphoryl
chloride in pyridine or other appropriate basic solvents.
The pyrimidine type 4-chloro groups or now displaced with
ammonium in methanol. Deprotection of the sugar hydroxyls
30 also takes place. The amino group is benzoylated by the
standard two-step process of complete benzylation (sugar
hydroxyls and amino group) and the acyls are selectively
removed by aqueous sodium hydroxide solution.
Alternatively, the *in situ* process of first treating the
35 nucleoside with chlorotrimethylsilane and base to protect

the sugar hydroxyls from subsequent acylation may be
employed. K. K. Ogilvie, *Can J. Chem.* 67:831-839 (1989).
Another conversion approach is to replace the pyrimidine
type 4-chloro group with an 1,2,4-triazolo group which

5   remains intact throughout the oligonucleotide synthesis on
the DNA synthesizer and is displaced by ammonium during the
ammonium hydroxide step which removes the oligonucleotide
from the CPG support and deprotection of the heterocycles.
Furthermore, in many cases the pyrimidine type 4-chloro

10   group can utilized as just described and replaced at the
end of the oligonucleotide synthesis.

**EXAMPLE 12 - Procedure for the attachment of 2'-deoxy-2'-
substituted 5'-dimethoxytriphenylmethyl ribonucleosides to
the 5'-hydroxyl of nucleosides bound to CPG support.**

15   The 2'-deoxy-2'-substituted nucleosides that will
reside in the terminal 3'-position of certain antisense
oligonucleotides is protected as their 5'-DMT (the cytosine
and adenine exocyclic amino groups are benzoylated and the
guanine amino is isobutyrylated) and treated with

20   trifluoro-acetic acid/bromoacetic acid mixed anhydride in
pyridine and dimethylaminopyridine at 50° C for five hours.
The solution is evaporated under reduced pressure to a thin
syrup which is dissolved in ethyl acetate and passed
through a column of silica gel. The homogenous fractions

25   were collected and evaporated to dryness. A solution of 10
ml of acetonitrile, 10 micromoles of the 3'-O-bromo-
methylester modified pyrimidine nucleoside, and one ml of
pyridine/dimethylaminopyridine (1:1) is syringed slowly (60
to 90 sec) through a one micromole column of CPG thymidine

30   (Applied Biosystems, INC.) that had previously been treated
with acid according to standard conditions to afford the
free 5'-hydroxyl group. Other nucleoside bound CPG columns
could be employed. The eluent is collected and syringed
again through the column. This process is repeated three

35   times. The CPG column is washed slowly with 10 ml of
acetonitrile and then attached to an ABI 380B nucleic acid

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synthesizer. Oligonucleotide synthesis is now initiated.
The standard conditions of concentrated ammonium hydroxide
deprotection that cleaves the thymidine ester linkage from
the CPG support also cleaves the 3',5' ester linkage

5   connecting the pyrimidine modified nucleoside to the
    thymidine that was initially bound to the CPG nucleoside.
    In this manner, any 2'-substituted nucleoside or generally
    any nucleoside with modifications in the heterocycle
    and/or sugar can be attached at the very 3'-end of an

10 oligonucleotide sequence.

    **EXAMPLE 13 - Procedure for the conversion of 2'-deoxy-2'-
    substituted   ribonucleoside-5'-DMT-3'-phosphoramidites into
    oligonucleotides.**

           The polyribonucleotide solid phase synthesis

15 procedure of B. S. Sproat, et al., *Nucleic Acids Research*
    17: 3373-3386 (1989) is utilized to prepare the 2'-modified
    oligonucleotides.

           Oligonucleotides of the sequence CGA CTA TGC AAG
    TAC having 2'-deoxy-2'-fluoro substituent nucleotides were

20 incorporated at various positions within this sequence. In
    a first oligonucleotide each of the adenosine nucleotides
    at positions 3, 6, 10, 11 and 14 (counted in a 5' to 3'
    directed) were modified to include a 2'-deoxy-2'-fluoro
    moiety. In a further oligonucleotide, the adenosine and

25 the uridine nucleotides at positions 3, 5, 6, 7, 10, 11, 13
    and 14 were so modified. In even a further
    oligonucleotide, the adenosine, uridine and cytidine
    nucleotides at positions 1, 3, 4, 5, 6, 7, 9, 10, 11, 13
    and 14 were so modified and in even a further

30 oligonucleotide, the nucleotides (adenosine, uridine,
    cytidine and guanosine) at every position was so modified.
    Additionally an oligonucleotide having the sequence CTC GTA
    CCT TCC GGT CC was prepared having adenosine, uridine and
    cytidine nucleotides at positions 1, 2, 3, 5, 6, 7, 8, 9,

35 10, 11, 12, 15 and 16 also modified to contain 2'-deoxy-
    2'-fluoro substituent moieties.

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Various oligonucleotides were prepared that
incorporated nucleotides having 2'-deoxy-2'-methylthio
substituents. For ascertaining the coupling efficiencies
of 2'-deoxy-2'-methylthio bearing nucleotides into
5   oligonucleotides, the trimer TCC and the tetramer TUU U
were synthesized. In the trimer, TCC, the central cytidine
nucleotide (the second nucleotide) included a 2'-deoxy-2'-
methylthio substituent. In the tetramer, each of the
uridine nucleotides included a 2'-deoxy-2'-methylthio
10   substituent. In further oligonucleotides, 2'-deoxy-2'-
methylthio substituent bearing nucleotides were
incorporated within the oligonucleotide sequence in
selected sequence positions. Each of the nucleotides at
the remaining sequence positions incorporated a 2'-O-methyl
15   substituent on its nucleotide. Thus all of the nucleotides
within the oligonucleotide included a substituent group
thereon, either a 2'-deoxy-2'-methylthio substituent or a
2'-O-methyl substituent. These oligonucleotides are: GAG
CUC CCA GGC having 2'-deoxy-2'-methylthio substituents at
20   positions 4, 5, 6, 7 and 8; CGA CUA UGC AAG UAC having 2'-
deoxy-2'-methylthio substituents at positions 1, 4, 5, 7, 9
and 13; UCC AGG UGU CCG AUC having 2'-deoxy-2'-methylthio
substituents are positions 1, 2, 3, 7, 9, 10, 11 and 14;
TCC AGG CCG UUU C having 2'-deoxy-2'-methylthio
25   substituents at positions 10, 11 and 12; and TCC AGG TGT
CCC C having 2'-deoxy-2'-methylthio substituents at
positions 10, 11 and 12.

**EXAMPLE 14 - Preparation of 2'-Deoxy-2'-fluoro Modified
Phosphorothioates Oligonucleotides.**
30   2'-Deoxy-2'-substituted 5'-DMT nucleoside
3'-phosphoramidites prepared as described in Examples 1-7
were inserted into sequence-specific oligonucleotide
phosphorothioates as described by S. Beaucage et al.,
Journal of American Chemical Society 112:1253-1255 (1990)
35   and B. S. Sproat, et al., *Nucleic Acids Research*
17:3373-3386 (1989).

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Oligonucleotides of the sequence CGA CTA TGC AAG
TAC having phosphorothioate backbone linkages and 2'-deoxy-
2'-fluoro substituent nucleotides were incorporated at
various positions within this sequence. In a first oligo-
5   nucleotide each of the backbone linkages was a
phosphorothioate linkage and each of the adenosine, uridine
and cytidine nucleotides at positions 1, 3, 4, 5, 6, 7, 9,
10, 11, 13 and 14 (counted in a 5' to 3' directed) were
modified to include a 2'-deoxy-2'-fluoro moiety. In a
10 further oligonucleotide each of the backbone linkages was a
phosphorothioate linkages and the nucleotides (adenosine,
uridine, cytidine and guanosine) at every position were
modified to include a 2'-deoxy-2'-fluoro moiety.

EXAMPLE 15 - Preparation of 2'-Deoxy-2'-fluoro Modified
15 **Phosphate Methylated Oligonucleotides.**
The protection, tosyl chloride mediated
methanolysis, and mild deprotection described by L. H.
Koole et al., in the *Journal of Organic Chemistry*
54:1657-1664 (1989), is applied to 2'-substituted
20 oligonucleotides to afford phosphate-methylated
2'-substituted oligonucleotides.

EXAMPLE 16 - Hybridization Analysis.
A. Evaluation of the thermodynamics of
hybridization of 2'- modified oligonucleotides.
25   The ability of the 2'- modified oligonucleotides
to hybridize to their complementary RNA or DNA sequences
was determined by thermal melting analysis. The RNA
complement was synthesized from T7 RNA polymerase and a
template-promoter of DNA synthesized with an Applied
30 Biosystems, Inc. 380B RNA species was purified by ion
exchange using FPLC (LKB Pharmacia,Inc.). Natural
antisense oligonucleotides or those containing
2'-modifications at specific locations were added to
either the RNA or DNA complement at stoichiometric
35 concentrations and the absorbance (260 nm) hyperchromicity

upon duplex to random coil transition was monitored using a
Gilford Response II spectrophotometer. These measurements
were performed in a buffer of 10 mM Na-phosphate, pH 7.4,
0.1 mM EDTA, and NaCl to yield an ionic strength of 10
5   either 0.1 M or 1.0 M. Data was analyzed by a graphic
representation of $1/T_m$ vs $ln[Ct]$, where $[Ct]$ was the total
oligonucleotide concentration. From this analysis the
thermodynamic para-meters were determined. Based upon the
information gained concerning the stability of the duplex
10 of heteroduplex formed, the placement of nucleotides
containing 2'-deoxy-2'-substituents into oligonucleotides
were assessed for their effects on helix stability.
Modifications that drastically alter the stability of the
hybrid exhibit reductions in the free energy (delta G) and
15 decisions concerning their usefulness as antisense
oligonucleotides were made.
          As is shown in the following Table 1, the
incorporation of 2'-deoxy-2'-fluoro nucleotides into
oligonucleotides resulted in significant increases in the
20 duplex stability of the modified oligonucleotide strand
(the antisense strand) and its complementary RNA strand
(the sense strand). In both phosphodiester backbone and
phosphorothioate backbone oligonucleotides, the stability
of the duplex increased as the number of 2'-deoxy-2'-fluoro
25 containing nucleotides in the antisense strand increased.
As is evident from Table 1, without exception, the addition
of a 2'-deoxy-2'-fluoro bearing nucleotide, irrespective of
the individual substituent bearing nucleotide or
irrespective of the position of that nucleotide in the
30 oligonucleotide sequence, resulted in a increase in the
duplex stability.
          In Table 1, the underline nucleotides represent
nucleotides that include a 2'-deoxy-2'-fluoro substituent.
The non-underlined nucleotides are normal nucleotides. The
35 oligonucleotides prefaced with the designation "ps" have a
phosphorothioate backbone. Unlabeled oligonucleotides are
normal phosphodiester backboned oligonucleotides.

TABLE 1.

EFFECTS OF 2'-DEOXY-2'-FLUORO MODIFICATIONS ON DNA(ANTISENSE) RNA(SENSE) DUPLEX STABILITY

	Antisense Sequence	G°37 (kcal/mol)	G°37 (kcal/mol)	Tm(°C)	Tm(°C)	Tm(°C)/subst
5	CGA CTA TGC AAG TAC	-10.11±0.04		45.1		
	CGA CTA TGC AAG TAC	-13.61±0.08	-3.50±0.09	53.0	+7.9	+1.6
	CGA CUA UGC AAG UAC	-16.18±0.08	-6.07±0.09	58.9	+13.8	+1.7
	CGA CUA UGC AAG UAC	-19.85±0.05	-9.74±0.06	65.2	+20.1	+1.8
	ps(CGA CTA TGC AAG TAC)	-7.58±0.06		33.9	-11.2	
10	ps(CGA CUA UGC AAG UAC)	-15.90±0.34	-8.32±0.34	60.9	27.0	+2.5
	CTC GTA CCT TCC GGT CC	-14.57±0.13		61.6		
	CUC GUA CCU UCC GGU CC	-27.81±0.05	-13.24±0.14	81.6	+20.0	+1.4

As is evident from Table 1, the duplexes formed between
RNA and an oligonucleotides containing 2'-deoxy-2'-fluoro
substituted nucleotides exhibited increased binding
stability as measured by the hybridization thermodynamic
5   stability. Delta Tm's of greater than 20°C were measured.
By modifying the backbone to a phosphorothioate backbone
even greater delta Tm's were observed. In this instance
delta Tm's greater than 31°C were measured. These fluoro
substituted oligonucleotides exhibited a consistent and
10   additive increase in the thermodynamic stability of the
duplexes formed with RNA. While we do not wish to be bound
by theory, it is presently believed that the presence of
the 2'-fluoro substituent results in the sugar moiety of
2'-fluoro substituted nucleotide assuming substantially a
15   3'-endo conformation and this results in the oligonucleo-
tide-RNA duplex assuming an A-type helical conformation.

B. Fidelity of hybridization of 2'-modified
oligonucleotides

The ability of the 2'- modified antisense oligo-
20   nucleotides to hybridize with absolute specificity to the
targeted mRNA was shown by Northern blot analysis of
purified target mRNA in the presence of total cellular RNA.
Target mRNA was synthesized from a vector containing the
cDNA for the target mRNA located downstream from a T7 RNA
25   polymerase promoter. Synthesized mRNA was electrophoresed
in an agarose gel and transferred to a suitable support
membrane (ie. nitrocellulose). The support membrane was
blocked and probed using [^{32}P]-labeled antisense
oligonucleotides. The stringency was determined by
30   replicate blots and washing in either elevated temperatures
or decreased ionic strength of the wash buffer.
Autoradiography was performed to assess the presence of
heteroduplex formation and the autoradiogram quantitated by
laser densitometry (LKB Pharmacia, Inc.). The specificity
35   of hybrid formation was determined by isolation of total
cellular RNA by standard techniques and its analysis by
agarose electrophoresis, membrane transfer and probing with

the labeled 2'-modified oligonucleotides. Stringency was
predetermined for the unmodified antisense
oligonucleotides and the conditions used such that only the
specifically targeted mRNA was capable of forming a
5   heteroduplex with the 2'-modified oligonucleotide.

C.   **Base-Pair Specificity of Oligonucleotides and RNA**
Base-pair specificity of 2-deoxy-2'-fluoro modified
oligonucleotides with the RNA complement (a "Y strand") was
determined by effecting single base-pair mismatches and a
10   bulge. The results of these determinations are shown in
Table 2. An 18 mer "X strand" oligonucleotide containing
14 adenosine, uridine and cytidine nucleotides having a 2'-
deoxy-2'-fluoro substituent was hybridized with the RNA
complement "Y strand" in which the 10 position was varied.
15   In Table 2, the underline nucleotides represent nucleotides
that include a 2'-deoxy-2'-fluoro substituent.

TABLE 2.

EFFECTS OF SINGLE BASE MISMATCHES ON 2'-DEOXY-2'-FLUORO MODIFIED DNA · RNA DUPLEX STABILITY

X strand: deoxy(CTC GTA CCT TTC CGG TCC)[3']
Y strand: ribo([3']GAG CAU GGY AAG GCC AGG[5'])

Y	base-pair type	$G°37$ (kcal/mol)	$G°37$ (kcal/mol)	$Tm(°C)$	$Tm(°C)$
A	Watson-Crick	-14.57±0.13		61.6	
C	T-C mismatch	-12.78±0.11	1.79±0.17	54.4	-7.2
G	T-G mismatch	-16.39±0.25	-1.82±0.28	61.7	0.1
U	T-U mismatch	-13.48±0.17	1.09±0.22	55.9	-5.7
none	bulged T	-14.86±0.35	-0.284±0.37	59.4	-2.2

X strand: deoxy(CUC GUA CCU UUC CGG UCC)[3']
Y strand: ribo([3']GAG CAU GGY AAG GCC AGG[5'])

Y	base-pair type	$G°37$ (kcal/mol)	$G°37$ (kcal/mol)	$Tm(°C)$	$Tm(°C)$
A	Watson-Crick	-27.80±0.05		81.6	
C	U-C mismatch	-21.98±0.28	5.82±0.28	73.8	-7.8
G	U-G mismatch	-21.69±0.16	6.12±0.17	77.8	-3.8
U	U-U mismatch	-18.68±0.15	9.13±0.16	73.6	-8.0
none	bulged U	-22.87±0.27	4.94±0.27	75.5	-6.2

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As is evident from Table 2, the 2'-deoxy-2'-fluoro modified oligonucleotide formed a duplex with the RNA complement with greater specificity than a like sequenced unmodified oligonucleotide.

5   **EXAMPLE 17 - Nuclease Resistance**
    A. Evaluation of the resistance of 2'- modified oligonucleotides to serum and cytoplasmic nucleases.
    Natural phosphorothioate, and 2- modified oligo-nucleotides were assessed for their resistance to serum
10 nucleases by incubation of the oligonucleotides in media containing various concentrations of fetal calf serum or adult human serum. Labeled oligonucleotides were incubated for various times, treated with protease K and then analyzed by gel electrophoresis on 20% polyacrylamine-urea
15 denaturing gels and subsequent autoradiography. Autoradiograms were quantitated by laser densitometry. Based upon the location of the modifications and the known length of the oligonucleotide it was possible to determine the effect on nuclease degradation by the particular
20 2'-modification. For the cytoplasmic nucleases, a HL60 cell line was used. A post-mitochondrial supernatant was prepared by differential centrifugation and the labeled oligonucleotides were incubated in this supernatant for various times. Following the incubation, oligo-nucleotides
25 were assessed for degradation as outlined above for serum nucleolytic degradation. Autoradiography results were quantitated for comparison of the unmodified, the phosphorothioates, and the 2'- modified oligonucleotides.
    Utilizing these test systems, the stability of a 15-mer
30 oligonucleotide having 2-deoxy-2'-fluoro substituted nucleotides at positions 12 and 14 and a phosphorothioate backbone was investigated. As a control, an unsubstituted phosphodiester oligonucleotide was 50% degraded within 1 hr and 100% degraded within 20 hours. In comparison for the
35 2'-deoxy-2'-fluoro substituted oligonucleotide having the phosphorothioate backbone, degradation was limited to less than 10% after 20 hours.

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B. Evaluation of the resistance of 2'- modified
oligonucleotides to specific endo- and exo-nucleases.
Evaluation of the resistance of natural and 2'-modified
oligonucleotides to specific nucleases (ie, endonucleases,
5   3',5'-exo-, and 5',3'-exonucleases) was done to determine
the exact effect of the modifications on degradation.
Modified oligonucleotides were incubated in defined
reaction buffers specific for various selected nucleases.
Following treatment of the products with proteinase K, urea
10   was added and analysis on 20% poly- acrylamide gels
containing urea was done. Gel products were visualized by
staining using Stains All (Sigma Chemical Co.). Laser
densitometry was used to quantitate the extend of
degradation. The effects of the 2'-modifications were
15   determined for specific nucleases and compared with the
results obtained from the serum and cytoplasmic systems.

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What is claimed is:

1.   A nuclease resistant oligonucleotide or
oligonucleotide analog for modulating the activity of a
selected sequence of RNA or DNA having a sequence of
nucleotide bases specifically hybridizable with said
5 selected sequence and having at least one modified 2'-
deoxyfuranosyl moiety.

2.   The oligonucleotide or oligonucleotide analog of
claim 1 wherein said modification comprises substitution by
H, OH, halo, azido, amino, substituted amino, cyano,
halomethyl, cyanato, alkoxyl, thioalkoxyl, haloalkoxyl,
alkyl sulfide, alkyl sulfonate, nitrate, nitrite, ammonium,
allyloxy or alkeneoxy.

3.   The oligonucleotide or oligonucleotide analog of
claim 1 wherein said modification comprises substitution by
H, OH, halo, azido, amino, allyloxy, methoxy, or alkyl.

4.   The oligonucleotide or oligonucleotide analog of
claim 1 having from about 5 to about 50 nucleotide bases.

5.   The oligonucleotide or oligonucleotide analog of
claim 1 wherein said modification is at the 3' end of said
oligonucleotide.

6.   The oligonucleotide or oligonucleotide analog of
claim 1 wherein at least one of the sugar linking groups is
replaced with carbon or ether linkages.

7.   The oligonucleotide or oligonucleotide analog of
claim 6 wherein a 5'-methylene group and carbocyclic sugar
are removed.

8.   The oligonucleotide or oligonucleotide analog of
claim 1 further modified such that at least some of the

sugar linking groups comprise a phosphorothioate, methyl phosphonate, or phosphate alkylate.

9.   The oligonucleotide or oligonucleotide analog of claim 1 in a pharmaceutically acceptable carrier.

10.   The oligonucleotide or oligonucleotide analog of claim 1 wherein said selected sequence of RNA or DNA comprises a portion of a HIV genome.

11.   The oligonucleotide or oligonucleotide analog of claim 1 wherein said selected sequence of RNA or DNA comprises a portion of a herpes virus genome.

12.   The oligonucleotide or oligonucleotide analog of claim 1 wherein said selected sequence of RNA or DNA comprises a portion of a papilloma virus genome.

13.   A method for modulating the production of a protein by an organism comprising contacting the organism with an oligonucleotide or oligonucleotide analog having a sequence of nucleotide bases specifically hybridizable with a
5   selected sequence of RNA or DNA coding for said protein, and having at least one modified 2'-deoxyfuranosyl moiety.

14.   The method of claim 13 wherein said modification comprises substitution by H, OH, halo, azido, amino, substituted amino, cyano, halomethyl, cyanato, alkoxyl,
10   thioalkoxyl, haloalkoxyl, alkyl sulfide, alkyl sulfonate, nitrate, nitrite, ammonium, allyloxy, or alkeneoxy.

15.   The method of claim 13 wherein said modification comprises substitution by hydrogen of a hydroxide, halo, azido, amino, methoxy, allyloxy or alkyl group.

15   16.   The method of claim 13 wherein said oligonucleotide has from about 5 to about 50 nucleotide bases.

17. The method of claim 13 wherein said modification is
at the 3' end of said oligonucleotide.

18.   The method of claim 1 wherein at least one of the
sugar linking groups of said modified oligonucleotide is
replaced with a carbon or ether linkage.

19. The method of claim 18 wherein a 5'-methylene group
and carbocyclic sugar are removed.

20. The method of claim 13 wherein the oligonucleotide
is further modified such that at least some of the sugar
linking groups of said oligonucleotide are modified to
comprise a phosphorothioate, methyl phosphonate, or
phosphate alkylate.

21. The method of claim 13 wherein the oligonucleotide
is in a pharmaceutically acceptable carrier.

22. The method of claim 13 wherein said selected
sequence of RNA or DNA comprises a portion of a HIV genome.

23. The method of claim 13 wherein said selected
sequence of RNA or DNA comprises a portion of a herpes
virus genome.

24. The method of claim 13 wherein said selected
sequence of RNA or DNA comprises a portion of a papilloma
virus genome.

25. A method for treating an organism having a disease
characterized by the undesired production of a protein
comprising contacting the organism with a nuclease
resistant oligonucleotide or oligonucleotide analog having
a sequence of bases specifically hybridizable with a RNA of
DNA sequence coding for said protein, wherein at least one

of the 2'-deoxyfuranosyl moieties of the nucleoside unit is modified, either alone or in combination with a pharmaceutically acceptable carrier.

26. The method of claim 25 wherein said modification comprises substitution by H, OH, halo, azido, amino, substituted amino, cyano, halomethyl, cyanato, alkoxyl, thioalkoxyl, haloalkoxyl, alkyl sulfide, alkyl sulfonate, nitrate, nitrite, ammonium, allyloxy or alkeneoxy.

27. The method of claim 25 wherein said modification comprises substitution by H, OH, halo, azido, amino, methoxy, allyloxy or alkyl.

28. The method of claim 25 wherein said oligonucleotide has from about 5 to about 50 nucleotide bases.

29. The method of claim 25 wherein said modification is at the 3' end of said oligonucleotide.

30. The method of claim 25 wherein at least one of the sugar linking groups of said modified oligonucleotide is replaced with a carbon or ether linkage.

31. The method of claim 30 wherein a 5'-methylene group and carbocyclic sugar are removed.

32. The method of claim 25 wherein at least some of the sugar linking groups of said modified oligonucleotide are modified to comprise a phosphorothioate, methyl phosphonate, or phosphate alkylate.

33. The method of claim 25 wherein said oligonucleotide is in a pharmaceutically acceptable carrier.

34. The method of claim 25 wherein said sequence of RNA or DNA comprises a portion of a HIV genome.

35. The method of claim 25 wherein said sequence of RNA or DNA comprises a portion of a herpes virus genome.

36. The method of claim 25 wherein said sequence of RNA or DNA comprises a portion of a papilloma virus genome.

37. A method for synthesizing 9-(2'-deoxy-2'-substituted-ß-D-ribofuranosyl)guanine from guanosine, comprising the steps of:
    oxidizing a 2'-hydroxyl residue of guanine to form oxidized guanosine;
    reducing said oxidized guanosine to yield 9-(ß-D-arabinofuranosyl)guanine;
    derivatizing the 2'-hydroxyl residue of said 9-(ß-D-arabinofuranosyl)guanine with a leaving group; and
    nucleophilically displacing said leaving group with inversion about the 2' position to yield 9-(2'-deoxy-2'-substituted-ß-D-ribofuranosyl)guanine.

38. The method of claim 37 wherein said reduction is effected at about -78°C.

39. The method of claim 37 wherein fluoride is used to displace said leaving group.

40. The method of claim 37 further comprising:
    protecting 3' and 5' positions of said guanosine with first protecting groups prior to said oxidation;
    protecting a N^2-amino residue of said 9-(ß-D-arabinofuranosyl)guanine with a second protecting group prior to said derivatization of said 2'-hydroxyl residue with said leaving group; and
    selectively deblocking 3' and 5' positions of said 9-(2'-deoxy-2'-substituted-ß-D-ribofuranosyl)guanine compound subsequent to said nucleophilic displacement.